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GRAS ASSOCIATES, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com

December 10, 2010

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Robert L. Martin

Re: GRAS Notification – High Purity Steviol Glycosides (≥95%)

Dear Dr. Martin:

On behalf of Sinochem Qingdao, Co. Ltd of Qingdao, China, we are submitting for FDA review a GRAS notification for High Purity Steviol Glycosides (≥95%). The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

(b) (6)

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

Enclosure: GRAS Notification – High Purity Steviol Glycosides (≥95%) (in triplicate)

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GRAS ASSOCIATES, LLC

GRAS ASSESSMENT

Of

HIGH PURITY STEVIOL GLYCOSIDES (≥ 95%)

Food Usage Conditions for General Recognition of Safety

For

SINOCHEM QINGDAO, Co., Ltd.
Qingdao, China

Evaluation by GRAS Expert Panel

Richard C. Kraska, Ph.D., DABT
Robert S. McQuate, Ph.D.
Madhusudan G. Soni, Ph.D., FACN

December 10, 2010

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

Sinochem Qingdao, Co., Ltd.'s ("Sinochem") high purity steviol glycosides, with rebaudioside A and stevioside as the principal components, which is referred to as SG 95 and by the tradename IceVia™, and which meets the specifications for high purity steviol glycosides (≥ 95%) as described below, has been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made by an appropriately convened panel of experts who are qualified by scientific training and experience; the GRAS evaluation is based on scientific procedures as described in the following sections; and the evaluation accurately reflects the conditions of the stevia-derived sweetener's intended use in foods.

Signed:

(b) (6)

12-10-2010

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR97702-3074

Date

B. Name &Address of Notifier

Sinochem Qingdao Co., Ltd.
The North Building, Golden Plaza
20 Xianggang Zhong Road
Qingdao, China 266071

As the notifier, Sinochem accepts responsibility for the GRAS determination that has been made for the high purity steviol glycosides as described in the subject notification; consequently, the high purity steviol glycosides preparations meeting the conditions described herein are exempt from premarket approval requirements for food ingredients.

[1] See 62 FR 18938 (17 April 1997) which is accessible at http://www.gpo.gov/fdsys/pkg/FR-1997-04-17/html/97-97-9706.htm.

C. Common Name & Identity of the Notified Substance

High purity steviol glycosides with rebaudioside A and stevioside as the principal components; also see Section III.A.

D. Conditions of Intended Use in Food

The high purity steviol glycosides preparations are intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into foods in general, other than in infant formulas and meat and poultry products, at per serving levels that reflect good manufacturing practices principles in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, the high purity steviol glycosides (≥ 95%) with rebaudioside A and stevioside as the principal components have been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR97702-3074.

II. INTRODUCTION

A. Objective

At the request of Sinochem, GRAS Associates, LLC ("GA") has undertaken an independent safety evaluation of Sinochem's high purity steviol glycosides preparations which are intended to be sold under the name of IceVia™. The preparations are composed of highly purified steviol glycosides with rebaudioside A and stevioside as the principal components. The purpose of the evaluation is to ascertain whether the intended food uses of high purity steviol glycosides (≥95%) as a general purpose non-nutritive sweetener are generally recognized as safe, i.e., GRAS, under the intended conditions of use as described in Section IV.A.

B. Foreword

Sinochem provided GA with substantial background information needed to enable the GRAS assessment to be undertaken. In particular, the information provided addressed the safety/ toxicity of steviol glycosides; the history of use of stevia in food; and compositional details, specifications, and method of preparation of the notified substance. Sinochem was asked to provide adverse reports, as well as those that supported conclusions of safety. Safety/toxicity studies performed with animals were noted to have value, along with available human testing. Sinochem was also asked to supply past and present human food use information. Knowing how much steviol glycosides has been safely consumed, i.e., the so-called "doses" or use levels, is critical in extrapolating to safe exposures for highly purified steviol glycosides when consumed as a food ingredient. The composite safety/toxicity studies, in concert with exposure information, ultimately provide the specific scientific foundation for the GRAS determination.

Sinochem supplied the requested safety/toxicity studies and consumption/exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through October 31, 2010. A GRAS assessment based primarily on the composite safety information, i.e., based on scientific procedures, was undertaken. Those references that were deemed pertinent to the objective at hand are listed in Section VIII.

C. Summary of Regulatory History of Stevia & Stevia-Derived Sweeteners

In South America and in several countries in Asia, including China, Japan, and Korea, stevia derived-sweeteners are permitted as a food additive. In recent years, the subject sweeteners have received food usage approvals in Mexico, Australia, New Zealand, and Switzerland. Steviol glycosides have been used as a dietary supplement in the US, since 1995 (Geuns, 2003). Based on the available information, no New Dietary Ingredient Notification for dietary supplement use of purified rebaudioside A has been made to the US FDA. Since 1989 and prior to 2008, at least two GRAS petitions seeking authorization for the addition of stevioside or steviol glycosides to

foods had been submitted to FDA. However, no authorizations had been issued by FDA in response to these filings, and subsequently these petitions were withdrawn. It appears that the previously available safety data---including purity considerations---for stevia, stevioside, or steviol glycosides were inadequate. Since 2008, the US FDA has issued "no questions" letters in response to the multiple GRAS notifications filed on Reb A and steviol glycosides.

Based on information from FDA's GRAS Notice Inventory[2] website as of December 9, 2010, the agency has received 15 notices on rebaudioside A or steviol glycosides. Eleven of these notices have received "no questions" letters from the FDA, while four notices are under agency review. In May 2008, Merisant and Cargill independently submitted GRAS notifications for rebaudioside A, highly purified forms of the steviol glycosides, to FDA. On December 17, 2008, FDA issued "no questions" letters for each of these GRAS notices. Since December 2008, a series of GRAS notifications were submitted to FDA for stevia-derived sweetener products by the following companies: McNeil Nutritionals, LLC; Blue California; Sweet Green Fields, LLC; Wisdom Natural Brands; Sunwin and WILD Flavors (two notifications); Pyure Brands, LLC, PureCircle USA, Inc., and GLG Life Tech Corp. Each of these firms received a "no questions" letter from FDA.[3] Additionally, two additional notifications submitted to FDA by GLG Life Tech Corp., one by NOW Foods and one from Guilin Layn Natural Ingredients Corp. are pending with the agency.

The Food Standards Australia New Zealand (FSANZ) has completed its evaluation of an application for use of steviol glycosides in foods in 2008. FSANZ recommended that the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) amend the Australia New Zealand Food Standards Code to allow the use of steviol glycosides in food (FSANZ, 2008).

The Joint Expert Committee on Food Additives (JECFA) has reviewed steviol glycosides at its 51st, 63rd, 68th and 73rdmeetings. In 2000, JECFA published the original review on steviol glycosides (WHO, 2000). JECFA established a temporary ADI (acceptable daily intake) of 0-2 mg/kg (on a steviol basis) at its 63rd meeting (WHO, 2006). Additionally, JECFA finalized food grade specifications (FAO, 2007a), although they were subsequently updated in 2008 (FAO, 2008) and 2010 (FAO, 2010) (see below). At the 69th meeting, the temporary status of the ADI was removed, and the ADI was raised to 0-4 mg/kg bw/day (on a steviol basis) as a result of the JECFA review of recently completed clinical studies with steviol glycosides (WHO, 2008). In 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009).

In early 2009, a number of parties, including the government of Australia and the Calorie Control Council, submitted a request to the Codex Committee on Food Additives in which it was proposed that the JECFA specifications for steviol glycosides should be modified to allow inclusion of

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[2] Accessible at: http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true.

[3] GRAS notification 252 was submitted by Merisant, GRAS notification 253 was submitted by Cargill, GRAS notification 275 was submitted by McNeil Nutritionals, GRAS notification 278 was submitted by Blue California, GRAS notification 282 was submitted by Sweet Green Fields, GRAS notification 287 was submitted by Wisdom Natural Brands; GRAS notifications 303 and 304 were submitted by Sunwin and Wild Flavors, GRAS notification 318 was submitted by Pyure Brands, GRAS notification 323 was submitted by PureCircle USA, and GRAS notification 329 was submitted by GLG Life Tech Corp; information pertaining to these notifications are listed on FDA's website at http://www.accessdata.fda.gov/scripts/fc/fcnNavigation.cfm?rpt=grasListing, along with their respective "no questions" letters.

Rebaudioside D and Rebaudioside F as specifically named acceptable glycosides that would be considered as part of the minimum 95% steviol glycosides composition (CCFA, 2009). This proposed modification was endorsed by the Codex Alimentarius Committee in July 2009; it was on the agenda for discussion at the JECFA Meeting in June, 2010 (FAO/WHO, 2009), and JECFA took final action in approving the modified steviol glycosides specifications to include Rebaudioside D and Rebaudioside F(FAO, 2010).

In 2008, Switzerland's Federal Office for Public Health (2008) approved the use of stevia as a sweetener citing the favorable actions of JECFA. Subsequently, France published its approval for the food uses of rebaudioside A with a purity of 97% (AFSSA, 2009).

Stevia-derived sweeteners are not permitted as an ingredient in conventional food in Hong Kong. This appears to be related to a lack of review of new data on the sweeteners rather than a safety concern. Although the government website cites permission to use stevia (Hong Kong Government, 2002), Hong Kong maintains that stevia is not permitted as a sweetener. The Hong Kong Government was reported to be waiting for the JECFA determination on the safety of steviol glycosides. However, no further official actions have been noted since JECFA's final resolution was reported in June 2008 or following subsequent JECFA actions in 2009 or 2010.

On September 18, 2009, based on a review of the international regulation of *Stevia rebaudiana* and the clinical evidence for safety and efficacy, the Natural Health Products Directorate, Health Canada (2009), has adopted the following guidelines for the use of stevia and steviol glycosides in Natural Health Products (NHPs). The revised recommendation for the maximum limit for steviol glycosides in NHPs is in accordance with the full ADI (acceptable daily intake) of 4 mg steviol/kg bw established by WHO (2008).

Earlier, the UK's Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food on September 24, 1998 rejected an application for use of steviol glycosides as a sweetener in herbal teas because "the applicant had not provided all of the information necessary to enable an assessment to be made."[4] In 1999, the Scientific Committee on Food for the European Commission concluded that "there are no satisfactory data to support the safe use of these stevia plants and leaves" (European Commission, 1999a).

In light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission for European Food Safety Authority (EFSA) to deliver a scientific opinion on the safety of steviol glycosides as a sweetener for use in the food categories specified in the dossiers from the three petitioners, EFSA reexamined the safety of steviol glycosides (EFSA, 2010).After considering all the data on stability, degradation products, metabolism and toxicology, the EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day, which is consistent with JECFA's determination.

[4] See http://www.maff.gov.uk/food/novel/980924.html.

D. FDA Regulatory Framework

Since 1995, steviol glycosides (or stevioside) have been used in dietary supplements in the US (Geuns, 2003). These supplements are widely available to consumers in the US through retail outlets and Internet purchases (Al-Achi and Greenwood, 2000). As established by FDA food regulation, dietary supplements cannot legally be added to conventional foods. In order to be incorporated into conventional foods, dietary supplements must undergo premarket approval by FDA as food additives or, alternatively, the ingredients must be determined to be generally recognized as safe (GRAS). The authority to make GRAS determinations is not restricted to FDA. In fact, GRAS determinations may be provided by experts who are qualified by scientific training and experience to evaluate the safety of food and food ingredients under the intended conditions of use.[5]

In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process. At that time, the petitioning process was replaced with a notification procedure.[6] While outlining the necessary content to be considered in making a GRAS determination, FDA encouraged that such determinations be provided to FDA in the form of a notification. However, notifying FDA of such determinations is strictly voluntary.

[5] See 21 CFR 170.3(i)(3).

[6] See 62 FR 18938 (17 April 1997) which is accessible at http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm.

III. CHEMISTRY & MANUFACTURE OF HIGH PURITY STEVIOL GLYCOSIDES & RELATED SINOCHEM PRODUCT

A. Common or Usual Name

High purity steviol glycosides is the common or usual name of the non-nutritive sweetener derived from *Stevia rebaudiana* Bertoni that is the subject of the GRAS evaluation. The compositional features of IceVia™, the subject high purity steviol glycosides (≥ 95%), are described in more detail in this section. JECFA adopted the term, steviol glycosides, for the family of chemical substances with sweetness properties that are derived from stevia. The term, stevia, is used more broadly to describe the plant or crude extracts of the plant.

B. Chemistry of Steviol Glycosides

The following chemistry related description of steviol glycoside is taken from the original JECFA monograph (WHO, 2000).

> Stevioside is a glycoside of the diterpene derivative steviol (ent-13-hydroxykaur-16-en-19-oic acid). Steviol glycosides are natural constituents of the plant *Stevia rebaudiana Bertoni*, belonging to the Compositae family. The leaves of *S. rebaudiana Bertoni* contain eight different steviol glycosides, the major constituent being stevioside (triglucosylated steviol), constituting about 5-10% in dry leaves. Other main constituents are rebaudioside A (tetraglucosylated steviol), rebaudioside C, and dulcoside A. *S. rebaudiana* is native to South America and has been used to sweeten beverages and food for several centuries. The plant has also been distributed to Southeast Asia. Stevioside has a sweetening potency 250-300 times that of sucrose and is stable to heat. In a 62-year-old sample from a herbarium, the intense sweetness of *S. rebaudiana* was conserved, indicating the stability of stevioside to drying, preservation, and storage(Soejarto et al., 1982; Hanson & De Oliveira, 1993).

The predominant sweetener components of stevia extracts have been identified as stevioside and Reb A. The chemical identities and key chemical identifiers for the two major components are presented in Table 1.

In the Chemical and Technical Assessment (FAO, 2007b), JECFA identified the sweetener components. They updated the list of common glycosides and their chemical structures, which are slightly different from compounds depicted in older publications (Nanayakkara et al., 1987; Suttajit et al., 1993). They are shown in Figure 1.

In a number of reviews by different authors (Kinghorn, 2002, Kennelly, 2002, Geuns, 2003), the structures of the components of steviol glycosides have been described. Through a series of chemical reactions and analyses the structures, stereochemistry, and absolute configurations of steviol and isosteviol were established over a 20-year period after the seminal work of Bridel and Lavielle (1931) in France. The work by Ogawa et al. (1980, cited in Brandle, et al., 1998) on synthetic transformation of steviol into stevioside supported the proposed structures. Two other

Table 1. Chemical Identity of Stevioside & Rebaudioside A

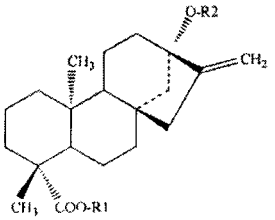
STEVIOSIDE	
Common name	Stevioside
Chemical name	13-[2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
Chemical formula	$C_{38}H_{60}O_{18}$
Formula weight	804.88
CAS Number	57817-89-7
REBAUDIOSIDE A	
Common Name	Rebaudioside A
Chemical name	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D- glucopyranosyl) oxy] kaur-16-en-18-oic acid, β-D- glucopyranosyl ester
Chemical formula	$C_{44}H_{70}O_{23}$
Formula weight	967.03
CAS Number	58543-16-1

sweet glycosides, Reb A and B, were obtained from methanol extracts of stevia leaves, along with the major sweet principle constituent, stevioside, and a minor constituent steviolbioside that which was first prepared from stevioside by alkaline hydrolysis by Wood et al. (1955; cited in Brandle et al., 1998). Subsequently, it was suggested that Reb B was an artifact formed from Reb A during isolation (Brandle et al., 1998; Kennelly, 2002). Furthermore, stevioside can be converted both chemically and enzymatically to Reb A. Further fractionation led to the isolation and identification of three other sweet glycosides respectively named Reb C, D, and E. It was reported that Reb A and D could be converted to Reb B by alkaline hydrolysis showing that only the ester functionality differed (Brandle et al., 1998). Dulcosides A and B were also described by Kobayashi et al. (1977). Subsequently, dulcoside B and Reb C were shown to be structurally identical.

C. Accepted Identity Specifications for Food Grade Steviol Glycosides

In addition to the manufacturing process, the composition of *Stevia rebaudiana Bertoni* extract depends upon the composition of the harvested leaves, which, in turn, is influenced by soil, climate, etc. (FAO, 2007b). As discussed in Section III.E., JECFA recommended that food grade specifications for steviol glycosides consist of a minimum of 95% on a dried weight basis of seven specific steviol glycosides (FAO, 2007a), and this has more recently been expanded to include

Figure 1. Chemical Structures of Various Steviol Glycosides[a, b]



	Compound name	C.A.S. No.	R1	R2
1	Steviol	471-80-7	H	H
2	Steviolbioside	41093-60-1	H	β-Glc-β-Glc(2→1)
3	Stevioside	57817-89-7	β-Glc	β-Glc-β-Glc(2→1)
4	Rebaudioside A	58543-16-1	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
5	Rebaudioside B	58543-17-2	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
6	Rebaudioside C (dulcoside B)	63550-99-2	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
7	Rebaudioside D	63279-13-0	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
8	Rebaudioside E	63279-14-1	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
9	Rebaudioside F	438045-89-7	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
10	Rubusoside	63849-39-4	β-Glc	β-Glc
11	dulcoside A	64432-06-0	β-Glc	β-Glc-α-Rha(2→1)

[a] From FAO, 2007b.
[b] The indicated C.A.S. No. for Rubusoside as reported in the cited reference is incorrect and should be 64849-39-4.

the original seven specific steviol glycosides plus Reb D and Reb F (FAO, 2010; see Appendix A). The component glycosides of particular interest for their sweetening property are stevioside and Reb A. In addition to the newly added Reb D and Reb F, the other five glycosides that are found at substantially lower levels in the preparations of steviol glycosides and recognized by JECFA consist of Reb C, dulcoside A, rubusoside, steviolbioside, and Reb B.

D. Manufacturing Processes

Manufacturing processes for stevia-derived sweeteners have been described in the published scientific and patent literature. Sinochem's manufacturing process for IceVia™ is specifically discussed in the following Section III.D.2.

1. Scientific & Patent Literature

Steviol glycosides are typically obtained by hot water or alcohols (ethanol or methanol) extraction of *Stevia rebaudiana* Bertoni leaves. This extract is a dark particulate solution containing all the active principles plus leaf pigments, soluble polysaccharides, and other impurities. In some processes, the "grease" from the leaves is removed before the extraction by employing solvents such as chloroform or hexane (Kinghorn, 2002). JECFA also cited that the typical manufacture starts with extracting leaves with hot water, and the aqueous extract is then passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with methanol to release the glycosides, and the product is recrystallized with methanol. Ion-exchange resins may be used in the purification process. The final product is commonly spray-dried. There are several extraction patents for the isolation of steviol glycosides. Kinghorn (2002) has categorized the extraction patents into those based on solvent, solvent plus a decolorizing agent, adsorption and column chromatography, ion exchange resin, and selective precipitation of individual glycosides. In recent patents, methods such as ultrafiltration, metallic ions, supercritical fluid extraction with CO_2 and extract clarification with zeolite have been employed.

2. Processing to Produce Sinochem's SG 95 High Purity Steviol Glycosides (≥ 95%)

The manufacturing process employed by Sinochem is fairly typical and similar to that yielding other related high purity stevia-derived sweetener products found on the market.

The source of Sinochem's steviol glycosides preparation is the leaves of the *Stevia rebaudiana* Bertoni plant. Sinochem has developed a state-of-the-art process for extracting steviol glycosides from the stevia leaf, and the detailed process is described in Appendix B. In brief, steviol glycosides are obtained by the extraction of stevia leaves with water. Leaves from different varieties of stevia plants are used for stevioside and rebaudioside A production. Ferrous sulfate and calcium hydroxide are added to the extract solution to facilitate precipitation. The extraction solution is passed through a filter press followed by adsorption onto resin; the glycosides are subsequently eluted with ethanol. The ethanol is partially evaporated and the concentrated extract is passed through an ion exchange resin to remove minerals and then through an ultrafiltration membrane. Subsequently, about half the water is removed by nanofiltration, and the product is then spray dried. The content of the steviol glycosides in the final Sinochem product is ≥ 95%.

The ethanol used in the purification process complies with FCC 5th Edition specifications, and the ion exchange resins used in the manufacturing comply with 21 CFR 173.25. Sinochem's IceVia™ is prepared in accordance with current Good Manufacturing Practices (cGMP) in Shandong Province, China.

E. Specifications for Sinochem's IceVia™ & Supporting Methods

Sinochem has adopted product specifications for its IceVia™ product to meet or exceed current JECFA recommendations. A comparison of the specifications provided by Sinochem for the final dried product and those from JECFA is presented in Table 2. An analytical report which quantitatively determines the steviol glycosides content of multiple batches of IceVia™ (or SG 95), along with details of the methodology and method validation, is included in Appendix C. This report demonstrates that the products meet the purity criteria. In addition, Appendix D consists of certificates of analysis indicating that five batches of production material meet all product specifications. Appendix E documents that a particular production batch contains no detectable pesticide residues.

F. Stability Data for Stevioside & Rebaudioside A

Steviol glycosides have been reported to be stable over the pH range 3-9 and can be heated at 100°C for 1 hour. However, at pH levels greater than 9, under these conditions it rapidly decomposes (Kinghorn, 2002). At pH 10 steviolbioside would be the major decomposition product produced from stevioside by alkaline hydrolysis (Wood et al., 1955). Chang and Cook (1983) investigated the stability of pure stevioside and Reb A in carbonated phosphoric and citric acidified beverages. Some degradation of each sweetening component after 2 months of storage at 37°C was noted. However, no significant change at room temperature or below following 5 months of storage of stevioside and 3 months of storage of Reb A was noted. Exposure to 1 week of sunlight did not affect stevioside, but resulted in approximately 20% loss of Reb A. Heating at 60°C for 6 days resulted in 0-6% loss of Reb A.

Extensive stability data of Reb A have been reported in the GRAS notices submitted by Cargill (2008) and Merisant (2008). Merisant (2008) conducted experiments with rebaudioside A as a powder, as a pure sweetener in solution, and as an ingredient in both cola-type and citrus carbonated beverages. In these investigations no degradation was detected when the powder was stored at 105°C for 96 hours. It was concluded that the powder was stable when stored for 26 weeks at 40±2°C with relative humidity of 75±5%. Both published and unpublished testing results from Merisant revealed that Reb A in carbonated citric acid beverages and phosphoric acid beverages did not significantly degrade during prolonged storage at refrigeration, normal ambient, or elevated ambient temperatures. Minimal loss of Reb A was detected after storage at 60°C, with considerable degradation noted after 13 hours at 100°C for carbonated beverage solutions and pure sweetener solutions (Merisant, 2008).

In the GRAS notification by Cargill (2008), extensive stability testing on Reb A as a powder under various storage conditions and under a range of pH and temperatures was reported. Additionally, in this notification Reb A stability in several representative food matrices at room temperature and elevated temperatures was also reported. Stability profiles were created for table top sweetener applications, mock beverages including cola, root beer and lemon-lime, thermally processed beverages, yogurt, and white cake. The results of stability testing revealed some degradation products that had not been detected in bulk Reb A. These degradation products were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana* Bertoni.

All the degradation products were found to share the same steviol aglycone backbone structure as found in stevioside and Reb A, but they differ by virtue of the glucose moieties present. The results of stability testing revealed that Reb A is stable in various food matrices following several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, Reb A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

Table 2. Specifications for Sinochem's IceVia™ or SG 95 High Purity Steviol Glycosides (≥ 95%)

PARAMETER	JECFA[a] SPECIFICATIONS	SPECIFICATIONS SINOCHEM SG 95	METHOD
PHYSICAL			
Appearance	White to light yellow powder	White, Off-white hygroscopic powder	Organoleptic
Flavor	Sweet	Sweet	Organoleptic
Sweetness Intensity compared to sucrose	200-300 x	200-300 x	GB8270-1999
Aroma	Odorless or slight characteristic odor	Sweet	Organoleptic
CHEMICAL			
Total Steviol glycosides (%)	≥ 95	≥ 95	JECFA HPLC as stevioside & rebaudioside A
Total metals (ppm)	NS	≤10	USP31 <231>
Arsenic (ppm)	≤ 1	≤ 1	ICP MS AOAC
Lead (ppm)	≤ 1	≤ 1	ICP MS AOAC
Ethanol (ppm)	≤5000	≤ 5000	GC-MS
Methanol (ppm)	≤200	≤ 200	GC-MS
Loss on drying	≤ 6	≤ 6.0	USP31 <731>
pH	4.5-7.0	4.5-7.0	USP29 <791> (1 in100 solution)
Residue on ignition (%)	≤ 1.0	≤ 1.0	USP31 <281>
MICROBIOLOGICAL			
Total Plate Count, CFU/g	NA	< 1000	USP31 <2021>
Yeast and Mold, CFU/g	NA	< 100	USP31 <2021>
E. Coli	NA	Negative	USP31 <2021>
Staphylococcus aureus	NA	Negative	USP31 <2021>
Salmonella sp.	NA	Negative	USP31 <2021>
Pesticides	NS	None detected	USP31 <467>

a Prepared at 73rd JECFA (2010); NS = not specified; NA = not applicable

Cargill (2008, published as Clos et al., 2008) also conducted photostability studies on the dry powder and mock beverages to ascertain Reb A behavior under defined conditions of fluorescent and near UV light exposure. Reb A was determined to be photostable under the defined conditions of analysis. The authors stated that the observation of better stability than in the work by Chang and Cook (1983) is due to differences in analytical methods. From the stability testing reported, it was concluded that Reb A is stable in various food matrices following several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, Reb A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

In addition to the above described stability reports for purified Reb A, in a GRAS notification by Sunwin (2010) on purified steviol glycosides with Reb A and stevioside as the principal components, stability was investigated using a 0.04% solution of Reb A 80% in acidic solutions between pH 2.81 and 4.18. In this study, the solutions were stored at 32°C for 4 weeks, and the Reb A content was determined at 1,2 and 4 weeks. Reb A 80% was found to be very stable at pH 3.17 and above. At pH 2.81, after 4weeks of storage under accelerated conditions only a 7% loss of Reb A was noted. Sunwin also studied the stability of Reb A 80% in simulated beverages using 0.1% citric acid (pH 3.2). The solutions were pasteurized and stored for 8 weeks at 4° and 32°C, and little difference in sweetness perception was found under these conditions.

The stability data in the scientific literature for stevioside, the JECFA report, and the extensive stability testing presented by Merisant, Cargill and Sunwin support the position that Sinochem's IceVia™ is well-suited for the intended food uses.

IV. INTENDED FOOD USES & ESTIMATED DIETARY INTAKE

Sinochem intends to use IceVia™ in a wide range of foods as a general sweetener replacement for sugar. There have been many scholarly estimates of potential dietary intake of replacement sweeteners, including steviol glycosides that have been published (FSANZ, 2008; Renwick, 2008; WHO, 2003) or submitted to FDA (Merisant, 2008). In a recent GRAS notification (GRN 301), a simplified estimate was proposed to and accepted by FDA, based on the estimates of exposure in "sucrose equivalents" (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90th percentile consumer of a sweetener which is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg/kg bw/day for any population subgroup. As noted in Table 2, the minimum sweetness intensity for IceVia™ is 200-fold that of sucrose. Therefore, the 90th percentile consumer of IceVia™ would consume no more than half this level or less than 5 mg/kg bw/day. Based on an estimate that IceVia™ consists of 40% steviol equivalents,[7] the consumption of steviol would be less than 2 mg/kg bw/day.

A. Intended Uses

The subject high purity steviol glycosides preparations with Reb A and stevioside as the principal components are intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener that is added into foods in general. Sinochem does not intend to incorporate its IceVia™ product into infant formulas or meat and poultry products. The high purity steviol glycosides with Reb A and stevioside as the principal sweetening components will function as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19). The use levels will vary by food category, but the levels are self-limiting due to organoleptic factors and consumer taste considerations. The amounts of high purity steviol glycosides to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation.[8]

B. Food Uses as Addressed by JECFA, Merisant& Cargill

As part of their safety deliberations, both JECFA and FSANZ reviewed estimates of possible daily consumption of mixed steviol glycosides. In addition, Merisant and Cargill estimated the consumption of Reb A in their submissions to FDA. Estimated maximum use levels in various foods as evaluated by JECFA are summarized in Table 3.

[7] Calculated by Expert Panel by multiplying by ratio of molecular weight of steviol to molecular weight of stevioside.
[8] See 21 CFR 182.1(b)(1).

Table 3. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents

Food Uses	Maximum Use Level Reported[a] (mg Steviol Glycosides / kg of food)	Maximum Use Level Calculated[b] (mg Steviol Equivalents / kg of food)
Desserts	500	200
Cold confectionery	500	200
Pickles	1000	400
Sweetcorn	200	80
Biscuits	300	120
Beverages	500	200
Yogurt	500	200
Sauces	1000	400
Delicacies	1000	400
Bread	160	64

[a] From WHO, 2006. [b] Calculated by Expert Panel by multiplying by ratio of molecular weight of steviol to molecular weight of stevioside.

Table 4. Proposed Uses & Levels of Rebaudioside A by Merisant[a]

Food Uses	Reb A (ppm)
Tabletop sweeteners	30,000[b]
Sweetened ready-to-drink teas	90-450
Fruit juice drinks	150-500
Diet soft drinks	150-500
Energy drinks	150
Flavored water	150
Cereals (oatmeal, cold cereal, cereal bars)	150

[a] Merisant, 2008. [b] Reb A content of sachet prior to dilution and not representative of "as consumed."

In the GRAS Notification by Merisant, expected levels of use for Reb A for various food applications were listed. Merisant utilized food consumption survey data from 2003-2004 NHANES to determine the estimated daily intake from the proposed uses of rebaudioside A. On a per user basis, the mean and 90th precentile daily consumption of rebaudioside A was estimated as 2.0 and 4.7 mg/kg bw/day, respectively. Specific food uses and use levels are given in Table 4. In its notification, Cargill (2008) utilized a different approach in estimating dietary intake figures for rebaudioside A when incorporated as a general sweetener in a broad cross-section of processed foods. Cargill considered that with a few minor exceptions rebaudioside A uses and use levels would be comparable to those of aspartame uses in the US. Using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 2008), Cargill performed a side-by-side consumption analysis for rebaudioside A versus aspartame.

C. Estimated Daily Intake

Sinochem intends to use IceVia™ in foods other than infant formulas and meat and poultry products as a general-purpose sweetener and as a table top sweetener. The very conservative consumer intake estimates provided by JECFA (as shown above in Table 3) were utilized to gauge the potential human exposures of the subject steviol glycosides in foods as reported in the US and in other countries. JECFA evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that was prepared for the European Commission by the Scientific Committee on Food. JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars, at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1. The intakes ranged from 1.3 mg/kg bw/day with the African diet to 3.5 mg/kg bw/day with the European diet.

JECFA also estimated the per capita exposure derived from disappearance (poundage) data supplied by Japan and China. The Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US. Table 5 summarizes the exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee.

JECFA concluded that the replacement estimates were highly conservative---that is, the calculated dietary exposure overestimates likely consumption---and that true dietary intakes of steviol glycosides (as steviol) would probably be 20 – 30% of these values or 1.0 - 1.5 mg/kg bw/day on a steviol basis. Similarly, FSANZ (2008) estimated steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario, which resulted in estimated exposures of 0.3 - 1.0 mg/kg bw/day for the mean and 90th percentile consumer, respectively. FSANZ examined consumption in other age groups and concluded there was no safety concern in children of any age. Merisant also calculated a dietary estimate for Reb A of 2.0 mg/kg bw/day for the average consumer and 4.7 mg/kg bw/day for a 90th percentile consumer. On a steviol equivalent basis, the Merisant estimates would be 0.7 and 1.6 mg/kg

Table 5. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)

ESTIMATE	EXPOSURE (mg/kg BW/DAY)
GEMS/Food (International)[a]	1.3 -3.5 (for a 60 kg person)
Japan, Per Capita	0.04
Japan, Replacement Estimate[b]	3
US, Replacement Estimate[b]	5

[a] WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme.
[b] These estimates were prepared in parallel to those for the international estimates; it was assumed that all dietary sugars in diets in Japan and the US would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200:1.

bw/day, respectively. In another review conducted on behalf of Cargill and included in their GRAS notification, the intake of rebaudioside A when used as a complete sugar replacement was estimated at 1.3 – 3.4 mg/kg bw/day when calculated as Reb A (Renwick, 2008).

In concert with the JECFA intake estimates, the anticipated human exposures as projected independently and with different approaches by both Merisant and Cargill in compiling their GRAS dossiers for Reb A (Merisant, 2008 and Cargill, 2008) tended to converge to yield estimated daily intakes (EDIs) in the range 0.4 - 1.6 mg/kg bw/day on a steviol basis or 1.3 - 4.7 mg/kg bw/day on a Reb A basis. The actual daily intakes of stevioside and Reb A from products such as IceVia™ offerings and from Merisant, Cargill, McNeil Nutritional, Blue California, Sweet Green Fields, Wisdom Natural Brands, Sunwin and WILD Flavors, Pyure Brands, PureCircle USA, GLG Life Tech Corp. and other manufacturers' and suppliers' products cannot yet be determined with accuracy.

The extent that stevia-based sweeteners will penetrate the US food supply and the extent the market will select mixed steviol glycoside products versus Reb A products is uncertain. Furthermore, many competing non-caloric sweeteners are currently available to consumers, which have been successful in the marketplace, most notably aspartame and sucralose.

Based on the above discussion, the intake estimates presented here are viewed as being conservative. When comparing these EDI assessments for steviol glycosides, we see that total daily consumption of the steviol glycosides and Reb A for defined food uses and as a general purpose sweetener is expected to be substantially less than the acceptable daily intake values discussed at length in Section VI.B.1.

D. Other Information on Human Exposure to Stevia

For about 20 years, consumers in Japan and Brazil, where stevia had been approved as a food additive, have been using stevia extracts as non-caloric sweeteners.[9] It is reported that 40% of the artificial sweetener market in Japan is stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999). Stevia usage as a dietary supplement is presently permitted in the US, Canada, Australia and New Zealand. It has been widely used in China and Japan in food and in dietary supplements. In the US, stevia is available in packets containing 60 - 90 mg steviol glycosides for home supplement uses, such as in beverages or other foods. It is estimated that sales of stevia in the US reached $45 million in 2005 (The Food Institute Report, 2006). No estimates are available on the daily consumption levels of steviol glycosides consumed in the US *via* dietary supplements in the form of capsules, soft gels, tablets, etc. In South America, stevia is commonly used as a treatment for type 2 diabetes (Hawke, 2003). However, elevated doses in the range of 1 gram per person per day or more were reported to be necessary to achieve this therapeutic effect (Gregersen et al., 2004).

[9] See Raintree NutritionTropical Plant Database (www.rain-tree.com/stevia.htm).

V. SAFETY DATA FOR STEVIOL GLYCOSIDES

A. Safety Data on Steviol Glycosides: Reviews by Expert Bodies & Other Scientists

As Sinochem's IceVia™ or SG 95 primarily contains Reb A and stevioside, the scientific data on each component are relevant to the present safety assessment. A number of reviewers have assessed the biological, toxicological and clinical data on stevia and steviol glycosides (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002). Additionally, the national and international regulatory agencies have thoroughly reviewed the safety of stevia and its glycosides. Most notably, over the years JECFA has evaluated stevia and steviol glycoside multiple times (WHO, 2000, 2006, 2007, 2008) and continues to do so (FAO/WHO, 2009). Recently FSANZ (2008) also evaluated steviol glycosides for use in food. The majority of these reviews primarily focused on mixtures of steviol glycosides. The early reviews tracked the development of better toxicology studies on purer samples of steviol glycosides. These reviews followed with keen interest whether effects of concern seen in various toxicology studies, such as the decrease in fertility with crude stevia preparations and the mutagenic activity of the principle metabolite steviol, would be manifest in comprehensive studies using modern test protocols with pure test materials. Additionally, JECFA encouraged the further elucidation of clinical effects on blood pressure and glucose metabolism in hypertensive and diabetic individuals, respectively, in comparison to normal human subjects. By 2006, sufficient favorable data were generated for JECFA to yield a temporary ADI, which was finalized at an elevated level in 2008. More details on the JECFA reviews are discussed in Section V.A.1. The key toxicology and clinical data on steviol glycosides (primarily stevioside), more recently developed data on Reb A, and data on the principle metabolite, steviol, as evaluated by JECFA and other reviewers are summarized in Sections V.B, V.C and V.D, respectively.

1. Summary of Interim & Final JECFA Reviews

Earlier at its 51st meeting, JECFA (WHO, 2000) expressed the following reservations about the safety data available at that time for steviol glycosides:

> The Committee noted several shortcomings in the information available on stevioside. In some studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was available on other constituents or contaminants. Furthermore, no studies of human metabolism of stevioside and steviol were available. In addition, data on long-term toxicity and carcinogenicity were available for stevioside in only one species. The mutagenic potential of steviol has been tested sufficiently only *in vitro.*

Subsequently, additional data were generated on the metabolism of steviol glycosides and submitted to JECFA. This information suggested that the common steviol glycosides are converted to steviol by intestinal bacteria and then rapidly converted to glucuronides that are excreted. The committee now had a molecular basis to become comfortable with studies on test materials, which consisted of variable composition but were relatively high purity mixtures of the common steviol glycosides. The new information also revealed that in *in vitro* studies steviol is mutagenic, while *in vivo* condition it is not mutagenic. The committee became convinced that purified steviol glycosides did not impair reproductive performance as did crude preparations of

stevia and that there were sufficient chronic studies in rats with adequate no observed effect levels (NOEL) that could support a reasonable acceptable daily intake (ADI) in the range of doses that would be encountered by the use of steviol glycosides as a sugar substitute. However, JECFA wanted more clinical data to rule out pharmacological effects at the expected doses. The following excerpt was taken from the report of the 63rd meeting (WHO, 2006):

> The Committee noted that most of the data requested at its fifty-first meeting, e.g., data on the metabolism of stevioside in humans, and on the activity of steviol in suitable studies of genotoxicity *in vivo*, had been made available. The Committee concluded that stevioside and rebaudioside A are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*.
>
> The NOEL for stevioside was 970 mg/kg bw/day in a long-term study (Toyoda et al., 1997) evaluated by the Committee at its fifty-first meeting. The Committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to about 12.5–25 mg/kg bw/day (equivalent to 5–10 mg/kg bw/day expressed as steviol). The evidence available at present was inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g., those with hypotension or diabetes).
>
> The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg/kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg/kg bw/day (or 383 mg/kg bw/day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. The Committee noted that this temporary ADI only applies to products complying with the specifications.
>
> The Committee required additional information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics.

In 2007, at its 68th meeting, JECFA (WHO, 2007) concluded that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008. Subsequently, sufficient data had been received by JECFA to revise and finalize food additive specifications for steviol glycosides (FAO, 2007a). The Chemical and Technical Assessment report written after the 2007 meeting, explained the Committee's thinking which resulted in flexibility in the identity specifications (FAO, 2007b).

> In response to the call for data on "stevioside" for the 63rd meeting of the Committee, submissions from several countries showed that the main components of the commercially available extracts of stevia are stevioside and rebaudioside A, in various amounts ranging from about 10-70% stevioside and 20-70% rebaudioside A. The information indicated that most commercial products contained more than 90% steviol glycosides with the two main steviol glycosides comprising about 80% of the material. The 63rd JECFA required that the summed content of stevioside and rebaudioside A was not less than 70% and established a minimum purity of 95% total steviol glycosides. Analytical data showed that most of the remaining 5% could be accounted for by saccharides other than those associated with the individual steviol glycosides.
>
> Noting that the additive could be produced with high purity (at least 95%) and that all the steviol glycosides hydrolyze upon ingestion to steviol, on which the temporary ADI is based, the 68th JECFA decided it was unnecessary to maintain a limit for the sum of stevioside and rebaudioside content. The Committee

> recognized that the newly revised specifications would cover a range of compositions that could include, on the dried basis, product that was at least 95% stevioside or at least 95% rebaudioside A.

In 2008, based on additional clinical studies, at its 69th meeting, JECFA finalized the evaluation of steviol glycosides (WHO, 2008) and raised the ADI to 0 – 4 mg/kg bw/day and removed the "temporary" designation. The summary of the Committee's key conclusions in the final toxicology monograph addendum (WHO, 2009) were stated as follows:

> From a long-term study with stevioside, which had already been discussed by the Committee at its fifty-first meeting, a NOEL of 970 mg/kg bw per day was identified. At its sixty-third meeting, the Committee set a temporary ADI of0–2 mg/kg bw for steviol glycosides, expressed as steviol, on the basis of this NOEL for stevioside of 970 mg/kg bw per day (383 mg/kg bw per day expressed as steviol) and a safety factor of 200, pending further information. The further information was required because the Committee had noted that stevioside had shown some evidence of pharmacological effects in patients with hypertension or with type 2 diabetes at doses corresponding to about 12.5–25.0 mg/kg bw per day(5–10 mg/kg bw per day expressed as steviol).
>
> The results of the new studies presented to the Committee at its present meeting have shown no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. The Committee concluded that the new data were sufficient to allow the additional safety factor of 2 and the temporary designation to be removed and established an ADI for steviol glycosides of 0–4 mg/kg bw expressed as steviol.
>
> The Committee noted that some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides, but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.

2. Summary of FSANZ Review of Steviol Glycosides

In 2008, FSANZ completed a review of the safety of steviol glycosides for use as a sweetener in foods. FSANZ concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic subjects at doses up to 11 mg/kg bw/day. The FSANZ review discussed the adequacy of the existing database and several new studies, including the clinical studies reviewed by JECFA in the summer of 2007, most notably the work of Barriocanal et al., which was later published in 2008.

In their draft document, FSANZ also indicated that the new data in humans provides a basis for revising the uncertainty factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore, FSANZ established an ADI of 4 mg/kg bw/day for steviol glycosides as steviol equivalents, derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study (FSANZ, 2008).

3. Summary of EFSA Review of Steviol Glycosides

On March 10, 2010, EFSA adopted a scientific opinion on the safety of steviol glycosides (mixtures that comprise not less than 95% of stevioside and/or rebaudioside A) as a food additive. Earlier---in 1984, 1989 and 1999---the Scientific Committee for Food (SCF) evaluated stevioside as a sweetener. At the time, the SCF concluded that the use of stevioside was "toxicologically not acceptable" due to insufficient available data to assess its safety. However, in light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission, EFSA reevaluated the safety of steviol glycosides as a sweetener. As both rebaudioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for both glycosides, the EFSA Panel agreed that the results of toxicology studies on either stevioside or rebaudioside A are applicable for the safety assessment of steviol glycosides. Considering the available safety data (*in vitro* and *in vivo* animal studies and some human tolerance studies), the EFSA Panel concluded that steviol glycosides, complying with JECFA specifications, are not carcinogenic, genotoxic, or associated with any reproductive/ developmental toxicity. The EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on the application of a 100-fold uncertainty factor to the NOAEL in the 2-year carcinogenicity study in the rat when administering 2.5% stevioside in the diet. This is equal to 967 mg stevioside/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day). Conservative estimates of steviol glycosides exposures both in adults and in children suggest that the ADI could possibly be exceeded by European consumers of certain ages and geographies at the maximum proposed use levels.

B. Safety Data on Stevioside & Stevia Extracts that are Predominantly Stevioside

This Section summarizes studies on stevioside or stevia extracts that were identified compositionally as predominantly stevioside. Related studies on Reb A are discussed in Section V.C.

1. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

Several studies in rats (Wingard et al., 1980; Nakayama et al., 1986; Koyama et al., 2003a) and other animal models, including chickens (Geuns et al., 2003a), hamsters (Hutapea et al., 1999), and pigs (Geuns et al., 2003b) indicate that stevioside is not readily absorbed from the GI tract. Available evidence from *in vitro* metabolism studies suggests that bacteria in the colon of rats and humans can transform various stevia glycosides into steviol (Gardana et al., 2003). Steviol was shown to be more readily transported with *in vitro* intestinal preparations than various steviosides (Geuns, 2003, Koyama et al., 2003b). Slow absorption of steviol was indicated by detection in the plasma of rats given oral stevioside (Wang et al., 2004). However, Sung (2002) did not detect plasma steviol following oral administration of steviosides to rats. In studies with human and rat liver extracts, Koyama et al. (2003b) demonstrated that steviol can be converted to various glucuronides .Excretion of metabolites of stevioside after oral doses has been shown in urine and feces in rats (Sung, 2002) and hamsters (Hutapea et al., 1999). Oral doses in pigs led to the detection of metabolites in feces but not in urine (Geuns et al., 2003b).

In a human study with 10 healthy subjects, Geuns et al. (2006) measured blood, urine and fecal metabolites in subjects that received 3 doses of 250 mg of purified stevioside (>97%) 3 times a day for 3 days. Urine was collected for 24 hours on day 3 and blood and fecal samples were also taken on day 3. Free steviol was detected in feces but not in blood or urine. Steviol glucuronide was detected in blood, urine and feces. Approximately 76% of the total steviol equivalents dosed were recovered in urine and feces. Based on these measurements, the authors concluded that there was complete conversion of stevioside in the colon to steviol, which was absorbed and rapidly converted to the glucuronide.

In a recent publication, Renwick and Tarka (2008) reviewed studies on microbial hydrolysis of steviol glycoside. The reviewers concluded that stevioside and Reb A are not absorbed directly and both are converted to steviol by gut microbiota in rats and in humans. This hydrolysis occurs more slowly for Reb A than for stevioside. Studies have shown that steviol-16,17 epoxide is not a microbial metabolite. The authors concluded that there is a single hydrolysis product and that toxicological studies on stevioside are relevant to the safety assessment for Reb A.

2. Acute Toxicity Studies

The LD_{50} studies of stevioside (purity, 96%) following administration of a single oral dose to rodents are summarized in Table 6. No lethality was seen within 14 days after administration, and no clinical signs of toxicity or morphological or histopathological changes were found, indicating that stevioside is relatively harmless.

Table 6. Acute Toxicity of Stevioside (Purity 96%) Given Orally to Rodents

SPECIES	SEX	LD_{50} (g/kg bw)	REFERENCE
Mouse	Male and Female	>15	Toskulkao et al. (1997)
Mouse	Male	> 2	Medon et al. (1982)
Rat	Male and Female	>15	Toskulkao et al. (1997)
Hamster	Male and Female	>15	Toskulkao et al. (1997)

3. Subchronic Toxicity Studies

In three published studies, subchronic toxicity of stevioside was investigated in rats following oral administration. In addition, a reproduction study in hamsters included subchronic phases on the F_0, F_1 and F_2 generations. These studies are summarized in Table 7. One of these studies was particularly important because it served as a range-finding study for two subsequent chronic studies. In this 13-week toxicity study, Fischer 344 rats (10/sex/group) were given doses of 0, 0.31, 0.62, 1.25, 2.5, or 5% in the diet (equivalent to 160, 310, 630, 1300, and 2500 mg/kg bw/day) to determine the appropriate doses for a two-year carcinogenicity study. None of the animals died during the administration period, and there was no difference in body-weight gain between the control and treated groups during administration or in food consumption in the latter

part of the study. The activity of lactic dehydrogenase and the incidence of single-cell necrosis in the liver were increased in all groups of treated males. The authors considered these effects to be nonspecific, because of the lack of a clear dose-response relationship, the relatively low severity, and their limitation to males. Other statistically significant differences in hematological and biochemical parameters were also considered to be of minor toxicological significance. The authors concluded that a concentration of 5% in the diet was a suitable maximum tolerable dose of stevioside for a two-year study in rats (Aze et al., 1991).

In earlier 3-month rat studies reviewed by Geuns (2003)---the sample purity, doses, strain of rat were not reported---a no effect level was determined to be in excess of 2500 mg/kg bw/day and 7% of the diet, apparently due to lack of effects at the highest dose tested in both studies (Akashi and Yokoyama, 1975).

Table 7. Summary of Subchronic Studies on Stevioside

Study	Animal Model/ Group Size	Test Material/ Sample Purity	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results and Remarks
Aze et al., 1991[a]	F344 rat/ 10 females and 10 males in each of 6 groups	Stevioside/ Not reported	0, 0.31, 0.62, 1.25, 2.5, 5% in diet/13 weeks	Not reported	No effects observed on mortality, body weight or food consumption. Clinical chemistry investigation revealed increased LDH levels and histopathological investigation indicated increased incidence of single-cell liver necrosis in all male treated groups, but not in a clear dose-response relationship. Investigators did not consider these changes to be treatment related due to the small magnitude and low severity of changes, the lack of a clear dose relationship and the limitation to males only. Organ weights, urine chemistry and gross necropsy not discussed. Authors concluded that 5% stevioside in diet is a tolerable dose for a 2 year study.
Yodyingyuad and Bunyawong, 1991[a]	Hamster/ four groups of 20 (10 male, 10 female)	Stevioside/ 90%	0, 0.5, 1.0, 2.5 g/kg bw/day/ duration unclear/ 3 months	2500	F_0, F_1 and F_2 generations in reproductive study were dosed for 90 days. Histological examination showed no effect at any dose. Weights of organs, blood analysis, urine chemistry and gross necropsy not discussed. The F_1 and F_2 hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents).
Mitsuhashi, 1976[b]	Rat (strain not reported)	Stevioside/ Not reported	Dietary concentrations up to 7%/ 3 months	Not reported	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy and histopathology not discussed.
Akashi and Yokoyama, 1975[b]	Rat (strain not reported)	Stevioside/ Not reported	Oral doses up to 2500 mg/kg bw/3 months	2500	No effects noted at all doses tested. Experimental details such as body weight, organ weight, blood analysis, urine chemistry, gross necropsy and histopathology not discussed.

[a] Abstract only. [b] As reported by Geuns, 2003.

4. Chronic Toxicity Studies

In three separate studies summarized in Table 8, chronic effects of stevioside have been studied. No treatment-related increase in tumor incidence was seen in any of these studies. In the most recent and well-documented study (additional study details were presented to JECFA in 2006), the apparent no observed adverse effect level (NOAEL) in F344 rats was the dietary level of 2.5% (test sample purity 96%, Toyoda et al., 1997). At 5% of the diet, statistically significant decreases in body weight, percent survival and kidney weight were noted. The author attributed these effects to various factors. The decrease in body weight was attributed to an inhibition of glucose utilization. The decrease in survival seemed to have been caused by an unusual late onset of large granular lymphocyte leukemia in high dose males. The authors reported that this tumor is rather common in F344 rats and that the overall incidence in male rats was actually within the historical control range experienced in the laboratory where studies were conducted. The authors attributed the decrease in kidney weight as probably due to a decrease in chronic inflammation found in the histopathological examination relative to control animals.

5. Reproductive & Developmental Toxicity Studies

The use of *S. rebaudiana* as an oral contraceptive has been reported by Indians in Paraguay (Planas and Kuc, 1968; Schvartzman et al., 1977). In experimental studies in rats, crude stevia leaf extract has been shown to inhibit fertility (Planas and Kuc, 1968). Reproductive toxicity studies have been conducted with orally administered purified stevioside as tabulated in Table 8, No effect on fertility or reproductive parameters was seen in a three-generation study in hamsters at doses up to 2500 mg/kg/day (Yodyingyuad and Bunyawong, 1991). There was an absence of statistically significant effects at doses up to 3% (equivalent to 3000 mg/kg bw/day; sample purity 96%; Mori et al., 1981). Similar results were observed in an additional rat study that was reviewed by Geuns (2003) where limited information is available in English (Usami et al., 1995). In a recent study, no effect on pregnancy or developmental parameters were observed in Swiss albino mice with stevioside or aqueous stevia extract at doses up to 800 mg/kg bw/day in female mice (Kumar and Oommen, 2008). Further details on these studies to the extent available are presented in Table 9.

6. Mutagenicity & Genotoxicity Studies

In a series of studies mutagenic and genotoxic effects of stevia and stevioside were investigated. These studies are summarized in Table 10. All studies were negative with the exception of a comet assay done in rats (Nunes et al., 2007a). The methodology used in this study and the resulting conclusions have been questioned (Geuns, 2007; Nunes et al., 2007b and 2007c; Williams, 2007; and Brusick, 2008).

Table 8. Summary of Chronic Toxicity Studies on Stevioside

STUDY	ANIMAL MODEL/ GROUP SIZE	TEST MATERIAL/ SAMPLE PURITY	DOSES / DURATION	AUTHOR ASSIGNED NOAEL (mg/kg bw/day)	RESULTS AND REMARKS
Toyoda et al., 1997	F344 rat/ 50 per sex per group	95.6% Stevioside	*Ad libitum* 0,2.5, 5% of diet/~24 months (104 weeks)	Author did not assign a NOAEL. (Mid-dose calculates to 970 in males; JECFA, 2006)	A significant decrease in survival rates in males receiving 5%. General condition, body weight, food intake, mortality, hematological, histopathological and organ weights were observed. Body weight gains dose-dependently decreased in both sexes. Kidney weights were significantly lower in 5% males and ovary, kidney and brain weights were significantly increased in 5% females. Tumors and non-neoplastic lesions found in all groups, and were not correlated to treatment. Conclusion was that stevioside is not carcinogenic under these experimental conditions.
Xili et al., 1992[a]	Wistar rat/ 45 per sex per group	85% Stevioside	0, 0.2, 0.6, 1.2 % of diet/24 months	794 (high dose)	After 6, 12 and 24 months five rats from each group were sacrificed for analysis. No effects observed on growth, food utilization, general appearance, mortality or lifespan. No changes in hematological, urinary or clinical biochemical values. Histopathological analysis showed that the neoplastic and non-neoplastic lesions were unrelated to the level of stevioside in the diet.
Yamada et al., 1985	F344 rat/ 70 per sex per group, 30 per sex per group in low-dose	95.2% Steviol glycosides (75% stevioside; 16% Reb A)	0.1, 0.3, 1% of diet/22 months for males, 24 months for females	550 (high dose)	At 6 and 12 months, 10 males and 10 females were sacrificed for analysis. General behavior, growth and mortality were same among groups throughout the experiment. At 6 months, protein urea was significantly increased in females, and blood glucose was increased in both sexes, although urinary glucose not detected. Weights of liver, kidney, heart, prostate and testes were increased in males at 6 months, and weight of ovaries was decreased in females in dose-dependent manner. Histopathological examination showed differences in various organs at 6 months that were unrelated to stevioside dose. These differences were not found at 12 months. Authors concluded that there were no significant changes after 2 years.

[a] Only abstract available.

7. Clinical Studies & Other Reports in Humans

In several studies, pharmacological and biochemical effects of crude extracts of stevia leaves and purified steviol glycosides have been investigated. The effects noted included glucose uptake, insulin secretion, and blood pressure (Geuns, 2003a). In South America, stevioside is used as a treatment for Type II diabetes. These effects were key concerns for JECFA. In 2006, JECFA summarized the available clinical studies of stevioside and further studies were recommended (WHO, 2006). Subsequently, several studies were conducted, and in 2009, JECFA reviewed these new studies (WHO, 2009). JECFA's summaries of the key studies are included below.

a. Studies Summarized in 2006

In a study by Curi et al. (1986), aqueous extracts of 5 g of *S. rebaudiana* leaves were administered to 16 volunteers at 6-hour intervals for three days, and glucose tolerance tests were performed before and after the administration. Another six volunteers were given an aqueous solution of arabinose in order to eliminate possible effects of stress. The extract increased glucose tolerance and significantly decreased plasma glucose concentrations during the test and after overnight fasting in all volunteers.

In a multi-center randomized, double-blind, placebo-controlled trial of hypertensive Chinese men and women (aged 28–75 years), 60 patients were given capsules containing 250 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 750 mg of stevioside per day (equivalent to 11 mg/kg bw/day as calculated by FSANZ, 2008) and followed up at monthly intervals for one year. Forty-six patients were given a placebo. After 3 months, systolic and diastolic blood pressure in men and women receiving stevioside decreased significantly, and the effect persisted over the year. Blood biochemistry parameters, including lipids and glucose, showed no significant changes. Three patients receiving stevioside and one receiving the placebo withdrew from the study as a result of side effects (nausea, abdominal fullness, dizziness). In addition, four patients receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment. These effects subsequently resolved, and the patients remained in the study (Chan et al., 2000).

In a follow-up multi-center randomized, double-blind, placebo-controlled trial was conducted in hypertensive Chinese men and women (aged 20–75 years), 85 patients were given capsules containing 500 mg of stevioside (purity not stated) three times per day, corresponding to a total intake of 1500 mg of stevioside per day (equivalent to 21 mg/kg bw/day, as calculated by FSANZ, 2008). Eighty-nine patients were given a placebo. During the course of study, three patients in each group withdrew. There were no significant changes in body mass index or blood biochemistry parameters throughout the study. In the group receiving stevioside, mean systolic and diastolic blood pressures were significantly decreased compared with the baseline, commencing from about 1 week after the start of treatment. After 2 years, 6 out of 52 patients (11.5%) in the group receiving stevioside had left ventricular hypertrophy compared with 17 of 50 patients (34%) in the group receiving the placebo ($p < 0.001$). Eight patients in each group reported minor side effects (nausea, dizziness and asthenia), which led two patients in each group to withdraw from the study. Four patients in the group receiving stevioside experienced abdominal fullness, muscle tenderness, nausea and asthenia within the first week of treatment. These effects subsequently resolved and the patients remained in the study (Hsieh et al., 2003).

In a paired cross-over study, 12 patients with Type II diabetes were given either 1 g of stevioside (stevioside, 91%; other stevia glycosides, 9%) or 1 g of maize starch (control group), which was taken with a standard carbohydrate-rich test meal. Blood samples were drawn at 30 minutes before and for 240 minutes after ingestion of the test meal. Stevioside reduced postprandial blood glucose concentrations by an average of 18% and increased the insulinogenic index by an average of 40%, indicating beneficial effects on glucose metabolism. Insulin secretion was not significantly increased. No hypoglycemic or adverse effects were reported by the patients or

Table 9. Summary of Reproductive Toxicity Studies on Steviol Glycosides

Study	Animal Model/ Group size	Test Sample Purity Stevioside (unless Otherwise Noted)	Doses / Duration	Author Assigned NOAEL (mg/kg bw/day)	Results and Remarks
Kumar and Oommen, 2008	Swiss albino mice/ 4 groups of 5 females	Not reported	500 and 800 mg/kg bw/15 days	800	Stevioside and stevia extract (purity and composition not reported) did not have any effect on reproductive parameters in mice when administered to female mice before or during pregnancy. No changes seen in number of implantations or uterine resorptions. No gross anatomical or histopathologic effects seen in 16-day embryos.
Usami et al., 1995[a]	Wistar Rat/4 groups of 25 or 26 pregnant rats	95.6%[b]	0, 250, 500, 1000 mg/kg bw/10 days	1000	Pregnant rats given doses of stevioside by gavage once a day on days 6-15 of gestation and were sacrificed on day 20 of gestation. Fetuses were examined for malformations in addition to maternal and fetal body weight, number of live fetuses, sex distribution, and numbers of resorptions or dead fetuses. No treatment-related effects observed. Authors concluded that orally administered stevioside is not teratogenic in rats.
Yodyingyuad and Bunyawong, 1991	Hamster/ 10 male, 10 female per group (40 total)	90%	0, 500, 1000, 2500 mg/kg bw/day/ duration unclear/ 3 months	2500	Males from each group were mated to females from respective dose group. Each female was allowed to bear 3 litters during the course of experiment. Stevioside had no effect on pregnancies of females at any dose. The F_1 and F_2 hamsters continued to receive stevioside (via drinking water for one month, then at same dose as parents); showed normal growth and fertility. Histological examination showed no effect on reproductive organs at any dose.
Oliveira-Filho et al., 1989[a]	Rat/number not reported	Not reported (Dried Stevia Leaves)	0 or 0.67 g dried leaves /ml, 2 ml twice per day/ 60 days	Not reported	Prepubertal rats (25-30 days old) tested for glycemia; serum concentrations of thyroxine; tri-iodothyroxine; available binding sites in thyroid hormone-binding proteins; binding of ^{3}H-methyltrienolone (a specific ligand of androgen receptors) to prostate cytosol; zinc content of prostate, testis, submandibular salivary gland, and pancreas; water content of testes and prostate; body-weight gain; and final weights of testes, prostate, seminal vesicle, submandibular salivary gland, and adrenal. Only difference due to treatment was seminal vesicle weight, which fell to 60% compared to control.
Mori et al., 1981	Rat/11 male, 11 female per group (44 total)	96%	0, 0.15, 0.75 or 3 % of feed/60 days	2000	Males given stevioside dose in diet for 60 days before and during mating with females who received same diet (as mated male) 14 days before mating and 7 days during gestation. No effect due to treatment on fertility or mating performance, and no effect of fetal development. Rats of each sex had slightly decreased body weight gain at highest dose with non-significant increase in number of dead and resorbed fetuses at highest dose.
Planas and Kuc, 1968[c]	Rat/14 per group (28 total)	Not reported (Crude stevia extract)	0 or 5% Crude stevia extract /18 days	Not reported	Extract given orally to adult female rats for 12 days, who were mated with untreated males during the last 6 days. Fertility reduced to 21% of fertility in control rats and remained reduced in a 50-60 day recovery. Histological examination, weights of organs, blood analysis, urine chemistry and gross necropsy not discussed.

[a] Only abstract available. [b] As reported by European Commission, 1999b.

observed by the investigators. Systolic and diastolic blood pressure was not altered by stevioside administration (Gregersen et al., 2004).

Table 10. Mutagenicity & Genotoxicity Studies on Stevia Extracts & Stevioside

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCEN-TRATION / DOSE	RESULT	REFERENCE
Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Stevioside	83	5 mg/plate[a] 1 mg/plate[b]	Negative	Matsui et al. (1996)
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	99	50 mg/plate	Negative[c]	Suttajit et al. (1993)
Forward mutation	*S. typhimurium* TM677	Stevioside	83	10 mg/plate	Negative[c]	Matsui et al. (1996)
Forward mutation	*S .typhimurium* TM677	Stevioside	NS	10 mg/plate	Negative[c]	Pezzuto et al. (1985)
Forward mutation	*S. typhimurium* TM677	Stevioside	NS	Not specified	Negative[c]	Medon et al. (1982)
Gene mutation	Mouse lymphoma L5178Y cells, TK- locus	Stevioside	NS	5 mg/mL	Negative[c,d]	Oh et al. (1999)
Gene mutation (umu)	*S. typhimurium* TA1535/pSK1002	Stevioside	83	5 mg/plate	Negative[c]	Matsui et al. (1996)
Gene mutation	*B. subtilis* H17 rec+, M45 rec-	Stevioside	83	10 mg/disk	Negative[c]	Matsui et al. (1996)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	83	8 mg/mL 12 mg/mL	Negative	Matsui et al. (1996)
Chromosomal aberration	Human lymphocytes	Stevioside	NS	10 mg/mL	Negative	Suttajit et al. (1993)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	85	12 mg/mL	Negative[a]	Ishidate et al. (1984)
DNA damage (comet assay)	Wistar rats; liver, brain and spleen	Stevioside	88.62	4 mg/L (estimated to be 80 - 500 mg/kg bw/day)in drinking water for 45 days	Positive in all tissues examined, most notably in liver	Nunes et al. (2007a)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevioside, 52; Reb A, 22	250 - 2000 mg/kg bw	Negative[e]	Sekihashi et al. (2002)
DNA damage (comet assay)	Male ddY mouse stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	Stevia	NS	2000 mg/kg bw	Negative[e]	Sasaki et al. (2002)
Micronucleus formation	ddY mouse bone marrow and regenerating liver	Stevioside	NS	62.5 - 250 mg/kg bw	Negative	Oh et al. (1999)
Mutation	*D. melanogaster* Muller 5 strain	Stevioside	NS	2% in feed	Negative	Kerr et al. (1983)

NS = Not specified. [a] Without metabolic activation. [b] As calculated by Williams, 2007. [c] With and without metabolic activation (source not specified in original monograph). [d] Inadequate detail available. [e] Sacrificed at 3 hours and 24 hours.

In a randomized, double-blind trial designed, 48 hyperlipidemic volunteers were recruited to investigate the hypolipidemic and hepatotoxic potential of steviol glycoside extract. The extract used in this study was a product containing stevioside (73 ± 2%), rebaudioside A (24 ± 2%) and

other plant polysaccharides (3%). The subjects were given two capsules, each containing 50 mg of steviol glycoside extract or placebo, twice daily (i.e., 200 mg/day, equivalent to 3.3 mg/kg bw/day assuming an average body weight of 60 kg), for 3 months. One subject from placebo group and three from treatment group failed to complete the study for personal reasons, not related to adverse reactions. At the end of the study, both groups showed decreased serum concentrations of total cholesterol and of low-density lipoproteins. Analyses of serum concentrations of triglycerides, liver-derived enzymes and glucose indicated no adverse effects. The authors questioned the subjects' compliance with the dosing regimen, in view of the similarity of effect between treatment and placebo (Anonymous, 2004a). In a follow-up study, 12 patients were given steviol glycosides extract in incremental doses of 3.25, 7.5 and 15 mg/kg bw/day for 30 days per dose. Preliminary results indicated no adverse responses in blood and urine biochemical parameters (Anonymous, 2004b).

b. Studies Summarized in 2009

In a short term study of stevioside in healthy subjects, 4 male and 5 female healthy volunteers (aged 21–29 years) were provided with capsules containing 250 mg stevioside (97% purity) to be consumed3 times per day for 3 days (Temme et al., 2004). Doses, expressed as steviol, were 288 mg/day or4.4 mg/kg bw/day for females and 3.9 mg/kg bw/day for males. Twenty-four hour urine samples were taken before dosing on day 1 and after dosing on day 3.Fasting blood samples were taken before dosing on day 1, and six samples were taken at different time points on day 3 after dosing. Fasting blood pressure measurements were taken before the first capsule and at six different time intervals after the first dose. Urine was analyzed for creatinine, sodium, potassium, calcium, and urea. Blood was analyzed for plasma glucose, plasma insulin, alkalinephosphatase, ALT, GPT, creatine kinase, and lactate dehydrogenase. The clinical analyses of blood, blood pressure, and urine showed no differences between samples taken before or after dosing.

In an unpublished double-blind, placebo-controlled trial study reviewed at the sixty-eighth JECFA meeting, 250 mg of a product containing 91.7% total steviol glycosides, including 64.5% stevioside and18.9% rebaudioside A, was administered to groups of type 1 (n = 8) and type2 diabetics (n = 15) and non-diabetics (n = 15) 3 times daily for 3 months. Control groups with the same number of subjects received a placebo. After 3 months, there were no significant changes in systolic or diastolic blood pressure, glycated haemoglobin (HbA1c), blood lipids, or renal or hepatic function. No adverse effects were reported. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Barriocanal et al., 2006, 2008). The Committee previously noted that this product did not meet the proposed specification of "not less than 95% steviol glycosides" and that the study was conducted in a small number of subjects.

A study of antihypertensive effects was conducted in previously untreated mild hypertensive patients with crude stevioside obtained from the leaves of *S. rebaudiana*. Patients with essential hypertension were subjected to a placebo phase for 4 weeks and then received either capsules containing placebo for 24weeks or crude stevioside at consecutive doses of 3.75 mg/kg bw/day (7 weeks), 7.5 mg/kg bw/day (11 weeks) and 15 mg/kg bw/day (6 weeks). Comparison of

patients receiving stevioside with those on placebo showed neither antihypertensive nor adverse effects of stevioside. This study was approved by the local ethics committee and met the requirements of the Declaration of Helsinki (Ferri et al., 2006). The product in this study also did not meet the proposed specification.

In a long-term, randomized, double blinded, placebo-controlled study, Jeppesen et al. (2006) investigated the efficacy and tolerability of oral stevioside in patients with type 2 diabetes. In this study, 55 subjects received 500 mg stevioside (purity unspecified) or placebo (maize starch) 3 times daily for 3 months. Compared with the placebo, stevioside did not reduce the incremental area under the glucose response curve and maintained the insulin response and HbA1c and fasting blood glucose levels.HbA1c is an indicator of mean glucose levels and is used in identifying effects on the control of diabetes. No differences in lipids or blood pressure were observed. It is not clear whether this study was approved by the local ethics committee or met the requirements of the Declaration of Helsinki (Jeppesen et al., 2006).

A placebo-controlled double-blind trial was carried out in 49 hyperlipidemic patients (aged 20–70 years, number of males and females not supplied) not undergoing treatment. The study was approved by the local ethics committee and complied with the principles of the Declaration of Helsinki. Individuals were divided into two groups, with 24 subjects receiving placebo capsules and 25 receiving capsules containing a dose of 50 mg steviol glycosides (70% stevioside, 20% Rebaudioside A), equivalent to 1.04 mg steviol/kg bw/day, using the mean body weight of the treatment group, 72.7 kg. Two capsules were taken before lunch and two before dinner each day for 90 days. Six subjects withdrew from the study, four in the placebo group and two in the test group. Self-reported adverse reactions were recorded, and fasting blood samples were taken at the end of the study and analyzed for ALT, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein(LDL), very low density lipoprotein (VLDL), and triglycerides. No effects of treatment on ALT, AST, or GGT were found . Decreases in the total cholesterol and LDL were observed in both the stevioside group and the placebo group, which were not treatment related. No adverse effects were observed (Cavalcante da Silva et al., 2006). The Committee noted at its sixty-eighth meeting that the product used in this study did not meet the proposed specification.

C. Safety Data on Rebaudioside A[10]

Only limited studies were available on Reb A during the JECFA reviews. Since 2008, several well-designed toxicology studies that followed the current regulatory and other guidelines for such studies have been reported on purified rebaudioside A, although it is uncertain whether or not these studies were considered by JECFA during its 2008 deliberations. These investigations included additional subchronic studies in rats and one in dogs, mutagenicity studies, reproduction

[10] Questions about the safety of rebaudioside A were previously raised by Huxtable (2002) and Kobylewski and Eckhert (2008). Their respective concerns, as well as opposing views supporting the safety of designated food uses of rebaudioside A expressed by Expert Panels, have been outlined in other GRAS notifications that were submitted to FDA. A more detailed account can be found in GRAS Notifications 278, 287, 303, and 304.

and developmental studies in rats, and comparative pharmacokinetic studies with stevioside in rats and humans, as well as additional clinical studies.

1. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

Three recently completed studies have shed light on the absorption and fate of Reb A in rats and humans. For comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, rebaudioside A, toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol were examined in rats (Roberts and Renwick, 2008). Orally administered single doses of the radio labeled compounds were extensively and rapidly absorbed with plasma concentration-time profiles following similar patterns for stevioside and rebaudioside A. Within 72 hours of administration, elimination of radioactivity from plasma was essentially complete. All plasma samples had similar metabolite profiles; the predominant radioactive component in all samples was steviol, with lower amounts of steviol glucuronide(s) and low levels of one or two other metabolites. Rebaudioside A, stevioside, and steviol were metabolized and excreted rapidly, with the majority of the radioactivity eliminated in the feces within 48 hours. Urinary excretion accounted for less than 2% of the administered dose for all compounds in both intact and bile duct-cannulated rats, and the majority of the absorbed dose was excreted *via* the bile. After administration of the compounds to intact and bile duct-cannulated rats, radioactivity in the feces was present primarily as steviol. The predominant radioactive compound detected in the bile of all cannulated rats was steviol glucuronide(s), indicating de-conjugation in the lower intestine. The authors concluded that the overall data on toxicokinetics and metabolism indicate that rebaudioside A and stevioside are handled in an almost identical manner in the rat after oral dosing.

In a randomized, double blind, cross-over study in healthy male subjects, Wheeler et al. (2008) assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of rebaudioside A and stevioside. Following administration of rebaudioside A or stevioside, steviol glucuronide appeared in the plasma of all subjects, with median T_{max} values of 12.00 and 8.00 hours post-dose, respectively. Steviol glucuronide was eliminated from the plasma, with similar $t_{1/2}$ values of approximately 14 hours for both compounds. Administration of rebaudioside A resulted in a significantly (approximately 22%) lower steviol glucuronide geometric mean C_{max} value (1472 ng/mL) than administration of stevioside (1886 ng/mL). The geometric mean AUC_{0-t} value for steviol glucuronide after administration of rebaudioside A (30788 ng*hr/mL) was approximately 10% lower than after administration of stevioside (34090 ng*hr/mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72-hour collection period, accounting for 59% and 62% of the rebaudioside A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that both rebaudioside A and stevioside were hydrolyzed to steviol in the gastrointestinal tract prior to absorption. The majority of circulatory steviol was in the form of steviol glucuronide indicating rapid first-pass conjugation prior to urinary excretion. Only a small amount of steviol was detected in urine (rebaudioside A: 0.04%; stevioside: 0.02%). The investigators concluded that rebaudioside A and stevioside underwent similar metabolic and elimination pathways in humans with steviol glucuronide excreted primarily in the urine and steviol in the feces. No safety

concerns were noted as determined by reporting of adverse events, laboratory assessments of safety or vital signs (Wheeler et al., 2008).

Another pharmacokinetic investigation was done as a toxicokinetic (TK) phase of a dietary study to determine the potential of rebaudioside A toxicity in rats at levels up to 2000 mg/kg bw/day(Sloter, 2008a). Rebaudioside A and total steviol were detected in peripheral blood of rats during daily administration of 2000 mg/kg bw/day of rebaudioside A at extremely low levels, with mean plasma concentrations of approximately 0.6 and 12 ug/mL, respectively. Estimates of absorbed dose for rebaudioside A and total steviol were approximately 0.02% and 0.06%, respectively, based on the amounts measured in urine collected over 24 hours in comparison to daily administered dietary dose to rats. Mean fecal rebaudioside A and measured hydrolysis products expressed as Total Rebaudioside A Equivalents compared to daily administered dose results in an estimate of percent of dose recovered ≈ 84%.

2. Subchronic Toxicity Studies

Recently, Curry and Roberts (2008) reported the results of two repeat dose studies of rebaudioside A in Wistar rats. The results of these investigations suggest that administration of rebaudioside A to Han Wistar rats at dietary concentrations of up to 100,000 ppm (9938 and 11,728 mg/kg bw/day for males and females, respectively) for 4 weeks or 50,000 ppm (4161 and 4645 mg/kg bw/day for males and females, respectively) for 13 weeks did not present any evidence of systemic toxicity. In the 4-week study, rebaudioside A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000 and 100,000 ppm to male and female rats. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In the 13-week study, Wistar rats were fed diets containing rebaudioside A at dietary concentrations of 0, 12,500, 25,000 and 50,000 ppm. In high-dose male and females groups, reductions in body weight gain attributable to initial taste aversion and lower caloric density of the feed were observed. Inconsistent reductions in serum bile acids and cholesterol were attributed to physiological changes in bile acid metabolism due to excretion of high levels of rebaudioside A *via* the liver. All other hepatic function test results and liver histopathology were within normal limits. No significant changes in other clinical pathology results, organ weights and functional observational battery test results were noted. Macroscopic and microscopic examinations of all organs were unremarkable with respect to treatment-related findings. The NOAEL in the 13-week toxicity study was considered to be 50,000 ppm or approximately 4161 and 4645 mg/kg bw/day in male and female rats, respectively (Curry and Roberts, 2008).

In another 90-day dietary admix toxicity study, effects of rebaudioside A (99.5% purity) at target exposure levels of 500, 1000 and 2000 mg/kg bw/day were tested in Crl:CD(SD) rats (Nikiforov and Eapen, 2008; Eapen, 2007). Each group consisted of 20/animals/sex. No treatment related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters were noted. There were no treatment-related macroscopic, organ weight or microscopic findings. Significantly lower body weight gains were noted in the 2000 mg/kg bw/day group in males but not females. At the end of the dosing period, the body weight in males was 9.1% lower than the control group. Due to the small magnitude of difference from the control group value, the investigators did not consider this result to be adverse. The decrease was most

likely due to the large proportion of the diet represented by the test material. The NOAEL was determined as ≥2000 mg/kg bw/day.

A 6-month dietary toxicity study in Beagle dogs (4/sex/group) was conducted to investigate the potential adverse effects of rebaudioside A (97.5% purity) at dosage levels of 0, 500, 1000 or 2000 mg/kg bw/day (Eapen, 2008). There were no unscheduled deaths during the course of the study. No treatment-related clinical observations were noted. Administration of rebaudioside A did not affect home cage, open field observations and functional observations and measurements. No differences in hematology findings, serum chemistry findings, or urinalysis findings between the groups were noted. Additionally, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. The investigators concluded that no systemic toxicity of rebaudioside A was observed at dosage levels up to 2000 mg/kg bw/day and the assigned NOAEL was ≥ 2000 mg/kg bw/day.

3. Reproductive & Developmental Toxicity Studies

In a two-generation reproductive toxicity study, rebaudioside A (97% purity) at 0, 7,500, 12,500, and 25,000 ppm was administered in diet to male and female Han Wistar rats (Curry, et al., 2008). Administration of rebaudioside A was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. Similarly, administration of rebaudioside A did not affect reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology in either the F_0 or F_1 generations. The survival and general condition of the F_1 and F_2 offspring, their pre-weaning reflex development, overall body weight gains, and the timing of sexual maturation, were not adversely affected by rebaudioside A treatment. The NOAEL for reproductive effects was 25,000 ppm and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm or 2,048 to 2,273 mg/kg body weight/day (the highest dose tested).

The results from two unpublished studies with rebaudioside A (Sloter 2008a, b) further support the above described findings from published studies. In a two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were fed either basal diet or the diet containing rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter 2008a). For the F_0 and F_1 generations rebaudioside A doses were 0, 500, 1000 and 2000 mg/kg/day. At initiation of study, F_0 animals were approximately 7 weeks of age. The test diet was offered to the offspring selected to become the F_1 generation following weaning [beginning on postnatal day (PND) 21]. The F_0 and F_1 males continued to receive rebaudioside A throughout mating, continuing through the day of euthanasia. The F_0 and F_1 females continued to receive rebaudioside A throughout mating, gestation and lactation until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females as evaluated by estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints. Both for parental systemic and reproductive toxicity a dose level ≥2000 mg/kg bw/day (highest dose administered) was assigned to be the NOAEL.

In an embryo/fetal developmental toxicity study in rats (Sloter, 2008b), effects of rebaudioside A administered via gavage was tested. Rebaudioside A administration did not affect intrauterine growth and survival, and there were no test article-related fetal malformations or developmental variations at any dosage level. In the absence of maternal or developmental toxicity a dose level ≥2000 mg/kg bw/day (highest dose administered) was considered to be the NOAEL for maternal and embryo/fetal developmental toxicity.

4. Mutagenicity & Genotoxicity Studies

The mutagenicity and genotoxicity data on Reb A have greatly increased recently. In a set of *in vitro* and *in vivo* genotoxicity assays covering mutation, chromosome damage and DNA strand breakage, rebaudioside A consistently and uniformly revealed negative results (Pezzuto et al, 1985; Nakajima, 2000a; Nakajima, 2000b; Sekihashi et al., 2002. These studies are critically reviewed by Brusick (2008). JECFA also reviewed an unpublished chromosome aberration assay of rebaudioside A in cultured mammalian cells (Nakajima, 2000a) and did not find increases in chromosome aberrations.

Additionally, FDA also reviewed three unpublished studies on rebaudioside A including a bacterial mutagenicity study (Wagner and Van Dyke, 2006), a mouse lymphoma study (Clarke, 2006) and a mouse micronucleus study (Krsmanovic and Huston, 2006) submitted by Merisant as part of the GRAS Notification. All three studies demonstrated lack of mutagenic or genotoxic activity. Additionally, Williams and Burdock (2009) also reported lack of genotoxicity in another set of published studies that included *in vitro* mutagenicity assays with *Salmonella, E. coli,* and mouse lymphoma cells. These investigators also reported lack of *in vitro* clastogenic effects in Chinese hamster V79 cells and the absence of *in vivo* effects in a mouse micronucleus assay and a rat study for unscheduled DNA synthesis. The key mutagenicity testing results for rebaudioside A are summarized in Table 11.

5. Clinical Studies

In a four week randomized, double-blind, placebo controlled trial, hemodynamic effects of rebaudioside A at a dose of 1000 mg/day rebaudioside A (97% purity) or placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) were investigated (Maki et al., 2008a). Subjects were predominantly female (76%, rebaudioside A and 82%, placebo) with a mean age of ~41 (range 18 to 73) years. At baseline, mean resting, seated SBP/DBP was 110.0/70.3 mm Hg and 110.7/71.2 mm Hg for the rebaudioside A and placebo groups, respectively. Compared with placebo, administration of rebaudioside A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-hour ambulatory blood pressure responses. The investigators concluded that consumption of 1000 mg/day of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.

In another trial, effects of 16 weeks of consumption of 1000 mg rebaudioside A (97% purity, n = 60) were compared to placebo (n = 62) in men and women (33-75 years of age) with type 2 diabetes mellitus (Maki, et al., 2008b). Changes in glycosylated hemoglobin levels did not differ

significantly between the rebaudioside A (0.11 ± 0.06%, mean ± standard error) and placebo (0.09 ± 0.05%; p = 0.355) groups. Similarly, no significant (p > 0.05 for all) changes from baseline for rebaudioside A and placebo, respectively, in fasting glucose (7.5 ± 3.7 mg/dL and 11.2 ± 4.5 mg/dL), insulin (1.0 ± 0.64 µU/mL and 3.3 ± 1.5 µU/mL), and Cpeptide (0.13 ± 0.09 ng/mL and 0.42 ± 0.14 ng/mL) were noted. No treatment related changes in blood pressure, body weight, and fasting lipids were noted. Rebaudioside A was well tolerated, and records of hypoglycemic episodes showed no excess versus placebo. Based on these results, the investigators suggested that chronic use of 1000 mg rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

D. Studies on the Principal Metabolite: Steviol

In a number of studies, steviol, the principal mammalian metabolite of stevioside, has been investigated for its safety. The results of these studies are summarized in the following sections.

1. Acute Toxicity Studies

The oral LD_{50} of steviol (purity, 90%) in male and female mice and rats was reported to be > 15 g/kg bw. In this study, only one of 15animals died within 14 days of administration. The LD_{50} values in hamsters given steviol orally were 5.2 g/kg bw in males and 6.1 g/kg bw in females. Histopathological examination of the kidneys revealed severe degeneration of the proximal tubular cells, and these structural alterations were correlated with increased serum blood urea nitrogen and creatinine. The authors concluded that the cause of death was acute renal failure (Toskulkao et al., 1997).

2. Developmental Toxicity Studies

Groups of 20 pregnant golden hamsters were given steviol (purity, 90%) at doses of 0, 250, 500, 750, or 1000 mg/kg bw/day (only 12 animals at the highest dose) by gavage in corn oil on days 6 - 10 of gestation. A significant decrease in body weight gain and increased mortality (1/20, 7/20, and 5/12) were observed at the three highest doses, and the number of live fetuses per litter and mean fetal weight decreased in parallel. Histopathological examination of the maternal kidneys showed a dose-dependent increase in the severity of effects on the convoluted tubules (dilatation, hyaline droplets). However, no dose-dependent teratogenic effects were seen. The NOEL was 250 mg/kg bw/day for both maternal and developmental toxicity (Wasuntarawat et al., 1998).

3. Mutagenicity & Genotoxicity Studies

In a number of studies mutagenicity and genotoxicity of steviol has been investigated. These studies reviewed by JECFA are summarized in Table 12.

Table 11. Mutagenicity & Genotoxicity Studies on Rebaudioside A

END-POINT	TEST SYSTEM	MATERIAL	PURITY (%)	CONCENTRATION / DOSE	RESULT	REFERENCE
Bacterial Mutagenicity	5 Salmonella strains with and without exogenous metabolic activation system	Reb A	99.5	1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate	No mutagenic response	Wagner and Van Dyke (2006)
Bacterial Mutagenicity	5 Salmonella strains and 1 E coli strain with and without exogenous metabolic activation system	Reb A		Up to 5000 µg per plate	No mutagenic response	Williams and Burdock (2009)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesisassay in the absence and presence of exogenous metabolic activation system	Reb A	99.5	Cloning conc. of 500, 1000, 2000, 3000, 4000 and 5000 µg/mL	No mutagenic or clastogenic response	Clarke (2006)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesisassay in the absence and presence of exogenous metabolic activation system	Reb A		Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Chromosome Aberration	Chinese Hamster V79 cells	reb A		Up to 5000 µg/mL		Williams and Burdock (2009)
Mouse Micronucleus	Micronucleus study consisted of 7 groups, each containing 5 male and 5 female ICR mice.	Reb A	99.5	500, 1000 and 2000 mg/kg bw	No increase in micronuclei formation	Krsmanovic and Huston (2006)
Mouse Micronucleus		Reb A		Up to 750 mg/kg bw	No increase in micronuclei formation	Williams and Burdock (2009)
Unscheduled DNA Synthesis	*In vivo* rat	Reb A		Up to 2000 mg/kg bw	No increase in unscheduled DNA synthesis	Williams and Burdock (2009)
DNA damage (comet assay)	*Male BDF1 mouse stomach, colon, liver*	Stevia extract	Stevio-side, 52%; Reb A, 22%	250 - 2000 mg/kg bw	Negative[a]	Sekihashi et al. (2002)
Chromosomal aberration	*CHL/IU Chinese hamster lung fibroblasts*	Reb A	NS	1.2 - 55 mg/mL	Negative[b]	Nakajima (2000a)
Micronucleus formation	*BDF1 mouse bone marrow*	Reb A	NS	500-2000 mg/kg bw per day for 2 days	Negative[c]	Nakajima (2000b)
Forward mutation	*S. typhimurium* TM677	Reb A	NS	10 mg/plate	Negative[b]	Pezzuto et al. (1985)

NS = Not specified. [a] Sacrificed at 3 hours and 24 hours. [b] With or without metabolic activation (source not specified in original monograph).
[c] Sacrificed at 30 hours after 2nd administration.

Table 12. Mutagenicity & Genotoxicity Studies on Steviol

STUDY	*IN VIVO/IN VITRO*	SYSTEM	TEST SAMPLE PURITY	AUTHOR CONCLUSION	RESULTS AND REMARKS
Sekihashi et al., 2002[a]	*In Vivo/In Vitro*	Comet Assay	Not reported	Negative	In *in vitro* study, steviol at 62.5, 125, 250 and 500 µg/ml did not damage DNA of TK6 and WTK1 cells in presence or absence of S9 mix. In *in vivo* study, mice sacrificed 3 or 24 hours after one-time oral administration of 250, 500, 1000 or 2000 mg/kg of steviol. Stomach, colon, kidneys, testis and liver DNA not damaged. An identical *in vivo* experiment with stevia extract performed, which also gave negative results.
Oh et al., 1999[b]	*In Vivo?*	Cell Mutation and DNA damage	Not reported	Negative	Steviol gave negative results for cell mutation and DNA damage in cultured cells.
Matsui et al., 1996[c]	*In Vivo?*	Mutagenicity and Chromosome aberration (Chinese hamster lung fibroblasts)	Not reported	Positive	Gene mutation and chromosomal aberration found in Chinese hamster lung fibroblasts after metabolic activation of steviol. In hamsters, several metabolites of stevioside found that have not been found in rats or humans. Therefore, experimental relevance should be questioned when hamsters are used.
Terai et al., 2002[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Steviol found to be mutagenic in Aroclor induced rat liver S9 fraction. 15-oxo-steviol found to be mutagenic at 10% level of steviol. Specific mutagenicity of lactone derivative in presence of S9 mixture 10x lower than that of derivative without S9 mixture.
Temcharoen et al., 1998[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Mutagenic effects of steviol and/or metabolites found in *S.typhimurium* TM677 by tranversions, transitions, duplications, and deletions at the guanine phosphoribosyltransferase (gpt) gene. Magnitude of increase of these mutations over the control not reported.
Klongpanich-pak et al., 1997[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Steviol and stevioside inactive in TA strains of *S. typhimurium, e. coli WP2, uvrA/PKM101* and rec assay using *B. subtilis* even when microsomal activated fraction present. Magnitude of increase of these mutations over the control not reported.
Matsui et al., 1996[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	Testing of Southern Blot technique with probe for gpt gene DNA of *E. coli.* The chromosomal DNA of TM677 and steviol-induced TM677 mutants digested by restriction enzymes and probed. No significant differences found in fragment length between wild-type and mutant DNA.
Matsui et al., 1996[a]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Both	Steviol weakly positive in umu test, either with or without metabolic activation. Steviol negative in reverse mutation and other bacterial assays even in presence of S9 activation.

STUDY	*IN VIVO/IN VITRO*	SYSTEM	TEST SAMPLE PURITY	AUTHOR CONCLUSION	RESULTS AND REMARKS
Procinska et al., 1991[c]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Negative	The direct mutagenic activity of 15-oxo-steviol was refuted.
Compadre et al., 1988[a]	*In Vitro*	Bacterial Mutagenicity, Mass Spec	Not Reported	Positive	Mass spectral analysis of steviol and analogues under conditions known to produce a mutagenic response. 15-oxo-steviol, a product of the metabolite, 15-alpha-hydroxysteviol was found to be direct-acting mutagen. Magnitude of increase over control in assay not discussed.
Pezzuto et al., 1985[d]	*In Vitro*	Bacterial Mutagenicity	Not Reported	Positive	Using *S. typhimurium* TM677 strain, steviol found to be highly mutagenic in presence of 9000 x g supernatant from livers of Aroclor 1254-pretreated rats. This mutagenicty dependent on pretreatment of rats with Aroclor and NADPH addition, as unmetabolized steviol was inactive. None of other metabolites tested was mutagenic. Authors concluded that structural features of requisite importance for the expression of mutagenic activity may include a hydroxy group at position 13 and an unsaturated bond joining the carbon atoms at positions 16 and 17.
Temacha-roen et al., 2000[c]	*In Vivo*	Micronucleus (rat)	90%	Negative	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.
Temacha-roen et al., 2000[c]	*In Vivo*	Micronucleus (mouse)	90%	Negative	Very high doses (8 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.
Matsui et al., 1996[a]	*In Vivo*	Micronucleus (mouse)	Not Reported	Negative	Steviol did not increase number of micronuclei observed in this study.
Temacha-roen et al., 2000[c]	*In Vivo*	Micronucleus (hamster)	90%	Negative	Very high doses (4 g/kg bw) given to rats did not induce micronucleus in bone marrow erythrocytes in male and female animals.

[a] Abstract only. [b] As reported in JECFA, 2006.[c] As reviewed by Geuns, 2003. [d] Full article.

VI. DISCUSSION OF GRAS CRITERIA & PANEL SAFETY FINDINGS

A. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[11]

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that:

> "...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."
>
> "General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information."[12]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following component elements:[13]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

[11] See 21 CFR 170.3(i).
[12] See 21 CFR 170.30(a).
[13] See Footnote 1.

The apparent imprecision of the terms "appreciable", "at the time" and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu 1988; Renwick 1990).

As noted below, this safety assessment to ascertain GRAS status for high purity steviol glycosides for the specified food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

B. Panel Findings on Safety Studies of Steviol Glycosides

Sinochem's IceVia™ or SG 95 product with a minimum steviol glycosides content of 95% as identified in the subject notification meets the JECFA specifications in that the glycosides constitute 95% or more of the dry weight. As seen from the specifications appearing in Table 2 and the chemical composition detailed in Appendices C and D, the product consists primarily of stevioside and Reb A. Both of these steviol glycosides have been extensively studied in clinical, pharmacokinetic and toxicological studies. A majority of these studies have been published in peer-reviewed journals. The Panel has reviewed the data on both glycosides as well as the data on steviol, the principal metabolite.

1. Safety Data on Stevioside & Stevia Extracts that are Predominantly Stevioside

Because of their sweetness characters, steviol glycosides are unique as they have viable uses as a non-nutritive sweetener in foods.[14] Periodic reviews by JECFA over the years document the progression in acquiring knowledge of the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple investigations with *in vivo* and *in vitro* models, which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably, the initially reported concerns related to the effects of stevia leaves or crude extracts on fertility were refuted by the well-designed reproductive studies with purified steviol glycosides. All other concerns failed to manifest themselves at the doses employed in the long-term rat studies.

[14] It has also been reported that steviol glycosides may have pharmacological properties, which can be used to treat certain disease conditions such as hypertension and Type 2 diabetes. Chatsudthipong and Muanprasat (2009) published a comprehensive review where they note that such therapeutic applications have not been firmly established as being due to steviol glycosides. The reviewers point out that the effects occur at higher doses than would be used for sweetening purposes. Furthermore, many effects noted in older studies may have been due to impurities in preparations that do not meet the contemporary purity specifications established by JECFA for use as a sweetener. If oral doses of steviol glycosides impart pharmacological effects, such effects would undoubtedly occur due to actions of the principle metabolite, steviol, but the pharmacological effects of steviol have not been comprehensively investigated.

As discussed in Section V.A.1, at its fifty-first meeting, JECFA reasoned that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), on which to base a temporary ADI with an adequate margin of safety. The Committee was satisfied that the lack of carcinogenic response in these well-conducted studies justified their conclusion that the *in vitro* mutagenic activity of steviol, buttressed by the evidence of rapid biotransformation and elimination of absorbed steviol, did not present a risk of carcinogenic effects *in vivo.* In addition, they concluded that all common steviol glycosides share the same basic metabolic and excretory pathways. Therefore, JECFA has concluded that high purity preparations of various steviol glycosides are safe to use as a non-nutritive sweetener. The additional clinical data subsequently presented allowed JECFA to establish a permanent ADI of 0 - 4 mg/kg bw/day (based on steviol equivalents), which translates to 0 -10 mg/kg bw/day for stevioside (as determined from the ratio of molecular weights of steviol and stevioside). The estimated consumption levels for stevioside containing sweeteners summarized in Section IV are comfortably within the JECFA ADI.

The Panel also noted in a 2007 study that DNA damage was seen in a variety of organs as assessed by comet assay in rats given drinking water containing 4 mg/mL steviol glycosides for up to 45 days (Nunes et al., 2007a). The methodology used in this study was questioned by several experts in the field (Geuns, 2007; Williams, 2007; Brusick, 2008). The Panel has reviewed the cited publications and agrees with the challenges made by these scientists thereby discounting the conclusions from the Nunes et al. (2007a) study.

The Panel has reviewed the findings from human clinical studies related to pharmacological effects. Regarding the clinical effects noted in humans, in order to corroborate the observations in these studies that these effects of steviol glycosides only occur in patients with either elevated blood glucose or blood pressure (or both), JECFA called for studies in individuals that are neither hypertensive nor diabetic (WHO, 2006). The new data presented to JECFA and also published by Barriocanal et al. (2008) demonstrate the lack of pharmacological effects of steviol glycosides at 11 mg/kg bw/day in normal individuals or approximately slightly more than 4 mg/kg bw on the basis of steviol equivalents. It is possible that JECFA may also have reviewed the preliminary results associated with the recently published clinical studies on rebaudioside A (Maki et al., 2008a, b). The Panel concludes that there will be no effects on blood pressure and glucose metabolism in humans at the doses of Reb A expected from its use in food as a non-nutritive sweetener.

JECFA's review also included anticipated dietary patterns and the use concentrations expected in various foods in order to calculate an estimated daily intake (EDI) (WHO, 2003, 2006). Based on the assumption of 100% substitution of steviol glycosides for sugar, an EDI of 5 mg/kg bw/day of steviol was calculated for US consumption. JECFA noted that the replacement estimates were highly conservative and that this calculated intake of steviol glycosides (as steviol) would more likely be 20–30% of these values. Except for the scenario developed by JECFA with 100% replacement of sugars by steviol glycosides, and as discussed in Section IV.C and summarized in Table 5, the highest dietary estimate for use in foods for Reb A is 4.7 mg/kg bw/day. The Panel agrees with the JECFA ADI of 4 mg/kg bw/day based on steviol equivalents, which corresponds to 10 mg/kg bw/day of stevioside, and 12 mg/kg bw/day for rebaudioside A and notes that the estimates as contained in Table 5 of anticipated dietary intake are below the ADI.

2. Safety Data on Rebaudioside A

In addition to the information on steviol glycosides discussed in the previous section, there are recent additional data specific to Reb A. Since July 2008, over ten papers describing the results of a comprehensive research program by different groups on Reb A have been published. These studies formed the basis of the Cargill GRAS notification (GRN 253, 2008). Several other studies were sponsored by Merisant, and these were also then submitted with their GRAS notification (GRN 252). Previously, only a limited number of toxicology studies and clinical studies on Reb A were conducted and reported. As in the previous section on steviol glycosides, JECFA had concluded, even before these new studies were completed, that seven common steviol glycosides are safe for use as sweetener preparations when present in any combination at a combined purity of 95% or more.

As a majority of the previous pharmacokinetic research was conducted with steviol glycosides, the presumed strategy adopted for the more recent research on Reb A was to conduct a limited number of well-designed and executed toxicology studies on Reb A itself and to demonstrate in rats and in humans that it is handled pharmacokinetically similarly to stevioside. These studies were also done to help justify using the JECFA-generated ADI (for steviol glycosides, expressed as steviol) without having to conduct a new chronic study in rats on Reb A. In addition, Merisant (see Table 11) as well as another group (Williams and Burdock, 2009) upgraded the mutagenicity and genotoxicity data available on Reb A with three assays that FDA reportedly believes are most predictive for carcinogenicity. The Cargill group also conducted two clinical studies to assure that Reb A does not have potentially adverse pharmacological effects on blood glucose and blood pressure as was previously demonstrated in some stevioside studies.

In two separate reviews by Carakostas et al. (2008) and Brusick (2008), the recent research on Reb A was summarized and combined with the body of knowledge on stevioside. These reviews summarized the findings of the Cargill research program as follows:

- Steviol glycosides, Reb A, and stevioside are not genotoxic *in vitro*.
- Steviol glycosides, Reb A, and stevioside have not been shown to be genotoxic *in vivo* in well-conducted assays.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes et al., 2007a) and was improperly interpreted as a positive response.
- Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.
- The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- The pharmacokinetic similarity between Reb A and stevioside justifies the use of the ADI established by JECFA---that was determined on studies employing stevioside as the main component---as the ADI for Reb A.

- The dietary levels expected from consumption of Reb A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.

Regarding possible pharmacological effects of Reb A in decreasing blood pressure and blood glucose, a recently published clinical study on Reb A addressed potential concerns (Maki et al., 2008a, b). The Panel has reviewed these clinical studies and concludes that there should be no effects on blood pressure and glucose metabolism in humans at the doses of Reb A expected from use in food as a non-nutritive sweetener.

C. Panel's Overall Conclusions

The Panel has critically reviewed the anticipated dietary patterns and the use concentrations expected for steviol glycosides in various foods considered by JECFA in order to calculate the EDI, and the Panel agrees with these assessments. For US consumption, based on the very conservative assumption of 100% substitution of steviol glycosides for all sugars, an EDI of 5 mg/kg bw/day steviol was calculated by JECFA. However, JECFA concluded that the replacement estimates were highly conservative and that this calculated intake of steviol glycosides (as steviol) would more likely be 20 - 30% of these values. Additionally, Renwick (2008) also concluded that if only Reb A were used as a total sugar replacement, the levels would be below the JECFA ADI. Moreover, FDA has recently concurred with a simpler approach to estimate the maximum consumption of high intensity sweeteners based on the sucrose equivalents (BioVittoria, 2009). Using this method, the Panel estimates that an EDI of 2 mg/kg bw/day steviol (see calculations in Section IV) very conservatively represents a potential high user of steviol glycosides if this non-nutritive sweetener becomes widely available in food. This is within a factor of two of the EDI of 1.0-1.5 mg/kg bw/day steviol calculated by JECFA.

The Panel recognizes that JECFA is composed of dozens of scientists that are internationally known experts on food ingredient safety that have established ADIs for food ingredients over the past 40 years. In addition to JECFA's safety assessment of steviol glycosides, both Merisant and Cargill took rather rigorous scientific approaches to demonstrate the safety of Reb A. The studies were equally well conducted. The safety profiles compiled by Merisant and Cargill differ somewhat, yet the results are complementary and are mutually reinforcing of rebaudioside A safety and, in turn, the safety of steviol glycosides. A series of pharmacokinetic studies with steviol glycosides and more recent data on Reb A demonstrate that Reb A is handled pharmacokinetically similarly to stevioside. Hence, the safety studies of Reb A are also applicable to the safety assessment of stevioside and other closely related steviol glycosides.

In consideration of the aggregate safety information available, the Panel concludes that JECFA has conducted an expert safety evaluation and agrees with JECFA's conclusion. The per person ADI for steviol glycosides of adequate purity as defined by JECFA specifications has been properly determined to be 4 mg/kg bw/day (as steviol equivalents). The Panel calculates that this is equivalent to 10 mg/kg bw/day for stevioside and 12 mg/kg bw/day for Reb A on a weight basis. The Panel agrees that adverse pharmacological effects are not likely to occur at this level

and that even high consumers of steviol glycosides are not likely to exceed this level. Therefore, the Panel agrees with the JECFA-derived ADI as a safe intake level of steviol glycosides and that food uses meeting the JECFA specifications, within the intake limits determined by JECFA, can be considered to be generally recognized as safe (GRAS) within the meaning of the Food, Drug, and Cosmetic Act.

The Panel considers the available qualitative and quantitative scientific evidence, including human and animal data, to be sufficient to establish safety-in-use with the designated ADI for high purity steviol glycosides. On the basis of scientific procedures,[15] the Panel concludes that the intended use of IceVia™ as a high purity steviol glycosides (≥ 95%) with Reb A and stevioside as the principal components, when added to food at levels up to full replacement of sugar on a sweetness equivalency basis, meets FDA's definition of safe.

D. Common Knowledge Elements for a GRAS Determination

The first common knowledge element for a GRAS determination requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The majority of the studies reviewed in this safety assessment have been published in the scientific literature as reported in Section V. Most of the literature relied upon by JECFA has also been published---most importantly, the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website. Thus, these studies become generally available to the scientific community. JECFA reviewed only a limited number of studies conducted specifically on Reb A. The collection of supporting data on Reb A has recently been enhanced by the publication of the 2008 studies. The newest clinical studies that address JECFA's concern with unwanted pharmacological effects due to steviol glycosides (Barriocanal et al., 2008) and with Reb A (Maki et al., 2008 a, b) have been published in the scientific literature.

To be sure, the Panel recognizes that the safety of steviol glycosides in human foods has been the subject of interest for many years. In addition to the reported substantial history of consumption of stevia, especially in South America and Asia, many scientific studies have been conducted and published. Some of the studies have raised safety concerns, and the Panel has given careful attention to such concerns. The overriding evidence, particularly with high purity steviol glycosides, has certainly diminished the Panel's concerns based on better study designs, better study execution, and new investigations that better reflect state-of-the-art toxicological and clinical principles and findings.

The remaining common knowledge element for a GRAS determination is that there must be a basis to conclude that there is consensus among qualified scientists about the safety of the substance with its intended use. The 2008 JECFA final opinion largely meets the common

[15] 21 CFR §170.3 Definitions.(h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

knowledge test on its own. The Panel is cognizant of the scientific rigor and broad base of scientific expertise that resides with the prestigious JECFA. JECFA is composed of expert scientists from various regulatory agencies around the world, as well as other scientists chosen because of their specific expertise on various classes of food ingredients. In addition, FDA participates in JECFA deliberations.

The JECFA conclusion has been reviewed and validated by other respected regulatory agencies, including FSANZ and the Switzerland Office of Public Health (FSANZ, 2008 and Switzerland Office of Public Health, 2008). A number of other well-respected scientists have indicated that steviol glycosides are safe for human consumption at doses in the range of the JECFA ADI (Xili et al., 1992; Toyoda et al., 1997; Geuns, 2003; Williams, 2007).

The common knowledge element has been embellished by the many well-respected scientists that participated in the Cargill-sponsored research conducted on Reb A, most notably David Brusick, Nigel Brown, and Andrew Renwick. An assertion of "general recognition of safety" was also made by Carakostas et al. (2008). We also note that the favorable safety conclusions on McNeil Nutritionals GRAS notification on steviol glycosides with Reb A as a principal component were reported by the McNeil Expert Panel, along with FDA's concurrence with Blue California's GRAS designation for its high purity Reb A. Similar safety conclusions were noted with FDA "no questions" letters as issued to Sweet Green Fields, Wisdom Natural Brands, Sunwin and WILD Flavors, Pyure Brands, PureCircle USA, and GLG Life Tech Corp. In summary, many diverse groups of scientists from all corners of the globe together provide strong fulfillment of the consensus requirement. Of particular significance from the perspective of establishing consensus for the safety of high purity steviol glycosides are the mid-December 2008 "no questions" determinations by FDA for the GRAS notifications for Reb A as submitted by Merisant and Cargill and the more recent comparable findings by FDA with the additional GRAS notifications cited above.

While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Panel believes that a wide and expanding consensus does exist in the scientific community to support a GRAS conclusion as outlined in this evaluation. The scientific community will undoubtedly conclude that concerns expressed by others over the years (Huxtable, 2002) are likely to be satisfied by newer data on more purified materials and the rigid specifications for purity published by JECFA for steviol glycosides, including Reb A. Most notably the concerns on effects of fertility with crude extract have been addressed with a number of reproductive effects in rats and hamsters with purer materials (Usami et al., 1995; Yodyingyuad and Bunyawong, 1991; Mori et al., 1981). Several chronic rat studies with sufficiently high no effect levels---most notably the study by Toyoda et al. (1997)---are available to set an acceptable ADI based on FDA tested review methodology. The recent clinical studies put to rest the concern that effects on blood pressure and blood glucose will be seen at the dietary levels expected (Barriocanal et al., 2008). There is also a wide consensus that the body of newer research on Reb A is sufficient to establish safety, as opposed to the small group of scientists that argue that more studies need to be done before the sweetener is made available in the US.

VII. CONCLUSIONS[16]

IceVia™, also referred to as SG 95 high purity steviol glycosides (≥ 95%), with the designated composition as established by Sinochem, which is produced in accordance with FDA Good Manufacturing Practices requirements, and which meets at a minimum the JECFA purity specifications for steviol glycosides is Generally Recognized As Safe when consumed as a general purpose non-nutritive sweetener as defined in the subject notification and within the JECFA ADI of 4 mg/kg bw/day on a steviol equivalent basis. In order to remain within the designated ADI, it is important to observe good manufacturing practices principles in that the quantity of a substance added to food should not exceed the amount reasonably required to accomplish its intended technical effect.

This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

(b) (6)

Richard C. Kraska, Ph.D., DABT
Chair

(b) (6)

Robert S. McQuate, Ph.D.

(b) (6)

Madhusudan G. Soni, Ph.D., FACN

DATE: December 10, 2010

[16] The detailed educational and professional credentials for two of the individuals serving on the Expert Panel can be found on the GRAS Associates website at www.gras-associates.com. Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Soni's curriculum vitae can be accessed at: http://www.soniassociates.net/Soni%20CV.pdf. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety. Each individual has previously served on multiple GRAS Expert Panels. Dr. Kraska served as Chair of the Panel.

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APPENDIX A

JECFA Steviol Glycosides Specifications & Analytical Method

Updated JECFA Specifications for Steviol Glycosides -- 2010

STEVIOL GLYCOSIDES

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

SYNONYMS INS no. 960

DEFINITION The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and the product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside which are generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

Chemical name

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

C.A S. number

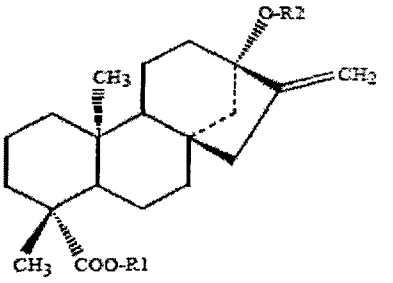
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: $C_{38}H_{60}O_{18}$
Rebaudioside A: $C_{44}H_{70}O_{23}$

Structural Formula

The nine named steviol glycosides:



Compound name	R1	R2
Stevioside	β-Glc	β-Glc-β-Glc(2→1)
Rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside C	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
Rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
Dulcoside A	β-Glc	β-Glc-α-Rha(2→1)
Rubusoside	β-Glc	β-Glc
Steviolbioside	H	β-Glc-β-Glc(2→1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides. Glc, Rha and Xyl represent, respectively, glucose, rhamnose and xylose sugar moieties.

Formula weight

Stevioside: 804.88
Rebaudioside A: 967.03

Assay	Not less than 95% of the total of the nine named steviol glycosides on the dried basis.
DESCRIPTION	White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.
FUNCTIONAL USES	Sweetener
CHARACTERISTICS	
IDENTIFICATION	
Solubility (Vol. 4)	Freely soluble in water
Stevioside and rebaudioside A	The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.
pH (Vol. 4)	Between 4.5 and 7.0 (1 in 100 solution)
PURITY	
Total ash (Vol 4)	Not more than 1%
Loss on drying (Vol. 4)	Not more than 6% (105°, 2h)
Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities").
METHOD OF ASSAY	Determine the percentages of the individual steviol glycosides by HPLC (Vol. 4) under the following conditions. Reagents Acetonitrile: more than 95% transmittance at 210 nm. Standards Stevioside: more than 99.0% purity on the dried basis. Rebaudioside A: more than 99.0% purity on the dried basis. Mixture of nine steviol glycosides standard solution: Containing stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and

steviolbioside. This solution is diluted with water-acetonitrile (7:3) accordingly and is used for the confirmation of retention times. Standards are available from Wako Pure Chemical Industries, Ltd. Japan and ChromaDex, USA.

Standard solution
Accurately weigh 50 mg of stevioside and rebaudioside A standard into each of two 50-ml volumetric flasks. Dissolve and make up to volume with water-acetonitrile (7:3).

Sample solution
Accurately weigh 50-100 mg of sample into a 50-ml volumetric flask. Dissolve and make up to volume with water-acetonitrile (7:3).

Procedure
Inject 5 µl of sample solution under the following conditions.
Column: Capcell pak C_{18} MG II (Shiseido Co.Ltd) or Luna 5µ C18(2) 100A (Phenomenex) or equivalent (length: 250 mm; inner diameter: 4.6 mm, particle size: 5µm)
Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L sodium phosphate buffer (pH 2.6)
Flow rate: 1.0 ml/min
Detector: UV at 210 nm
Column temperature: 40°
Record the chromatogram for about 30 min.

Identification of the peaks and Calculation
Identify the peaks from the sample solution by comparing the retention time with the peaks from the mixture of nine steviol glycosides standard solution (see under figure). Measure the peak areas for the nine steviol glycosides from the sample solution. Measure the peak area for stevioside and rebaudioside A from their standard solutions.
Calculate the percentage of each of the eight steviol glycosides except rebaudioside A in the sample from the formula:

$$\%X = [W_S/W] \times [f_X A_X/A_S] \times 100$$

Calculate the percentage of rebaudioside A in the sample from the formula:

$$\%Rebaudioside\ A = [W_R/W] \times [A_X/A_R] \times 100$$

where
X is each steviol glycoside;
W_S is the amount (mg) calculated on the dried basis of stevioside in the standard solution;
W_R is the amount (mg) calculated on the dried basis of rebaudioside A in the standard solution;
W is the amount (mg) calculated on the dried basis of sample in the sample solution;
A_S is the peak area for stevioside from the standard solution;
A_R is the peak area for rebaudioside from the standard solution;

A_X is the peak area of X for the sample solution; and
f_X is the ratio of the formula weight of X to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

Calculate the percentage of total steviol glycosides (sum the nine percentages).

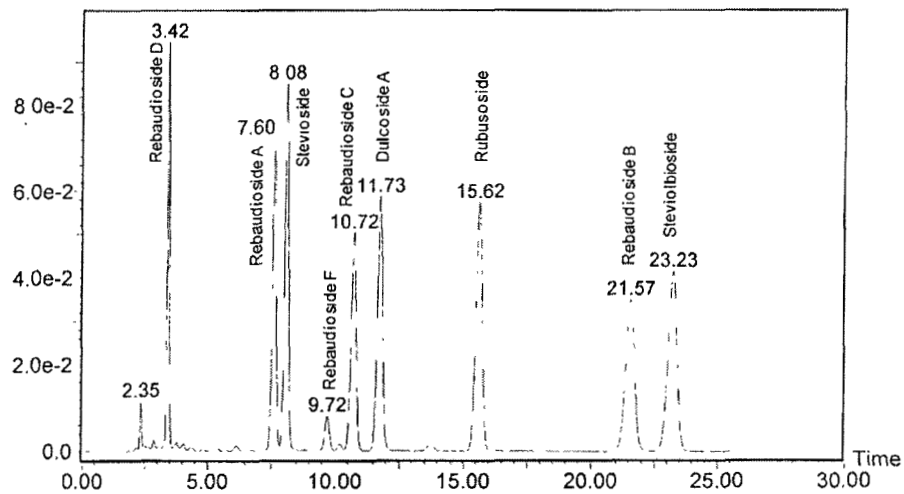


Figure. Chromatogram of mixture of nine steviol glycosides standard solution
Column: Capcell pak C_{18} MG II
Concentration: 0.5 mg/ml each except rebaudioside F (about 0.1 mg/ml)

APPENDIX B

Sinochem Manufacturing Information for Production of High Purity Steviol Glycosides (≥ 95%)

SINOCHEM QINGDAO CO., LTD.
20 Xianggangzhong Road, Qingdao 266071, China

SINOCHEM QINGDAO CO.,LTD.

Manufacturing Process for Steviol Glycosides

Production steps	Objective of the Process	Operating Condition
1. Stevia leaves Preparation	To remove freign matters (such as stevia plant stem and twigs or sand from stevia harvesting or stevia leaves packing) from stevia leaves.	Screening sieve
2. Water Extraction	To unlock the steviol glycosides from stevia leaves, and get the 1st stage syrup.	Stevia leaves : water = 1:20 w/v, and extract 6-7 times in non-ion water.
3. Flocculation	To precipitate the plant substances (such as pectin, pigment) from the 1st stage syrup.	Adding FeSO4 and CaO into the 1st stage syrup.
4. Filtration	To remove the precipitation from the 1st stage syrup, and get the 2nd stage syrup.	Filter Press
5. Filtration	To further remove plant impurties from the 2nd stage syrup, and get the 3rd stage syrup.	Micro Filter
6. Adsorption on Resin	To adsorb steviol glycosides on adsorption resin, and then release with ethanol, and get the 4th stage syrup	To have the 3rd stage syrup flow through adsorption resin, and then release the steviol glycosides with 70% ethanol.
7. Distillation	To recover the ethanol	Ethanol vaporisation at 80~100℃
8. Ion Exchange	To remove minerals, heavy metals and pigment from 4th stage syrup, and get the 5th stage syrup.	Cation resin and anion resin.
9. Membrane Purification	To further purify the 5th stage syrup, and get the 6th stage syrup.	UF membrane filtration.
10. Concentration	To remove 50-60% water from the 6th stage syrup, and get the concentrated clean syrup.	NF membrane filtration
11. Spray Drying	To spray the concentrated clean syrup into steviol glycosides powder.	Spray drying at high temperature.

APPENDIX C

Analyses of Multiple Production Lots of Sinochem's SG 95 High Purity Steviol Glycosides (≥ 95%)

Sinochem Qingdao Food Technology Center No. STV-001

Testing Procedure

Assay of Stevia

QA Manager Approval: Wang Zhenyang
R&D Chemist Approval: Jin Yan
Date: June 19, 2006
Updated: Jan 25, 2010

Materials and Reagents

Stevioside Standard, min.99.0%, Wako, Lot CDP6588
Rebaudioside A Standard, min.99.0%, Wako, Lot CDP6587
Acetonitrile, HPLC grade
Water, HPLC grade
Phosphoric acid, AR grade
0.45 micron membrane filter

Instrument and Condition

Analytical balance, Mettler-Toledo XS204
Chromatographic system: Aglient 1200, with an auto sampler and a degasser
Column: Kromasil 100-5NH2, 250mm x 4.6mm
Flow rate: Adjust the retention time of rebaudioside A to around 21 min
Mobile Phase: A 80:20 mixture of acetontrile and water, pH 3.0
Detect wavelength: 210 nm
Inject volume: 10 μl
Column temperature: 40℃

Preparation of Mobile Phase

Mix HPLC grade acetonitrile and water (80:20, v/v). Adjust the pH to 3.0 with phosphoric acid. Filter through 0.45 micron membrane filter.

Standard Preparation

Accurately weigh about 25 mg of dried Stevioside Standard (105℃ for 2h) into a 25-ml. volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
Accurately weigh about 25 mg of dried Rebaudioside A Standard (105℃ for 2h) into a 25-mL volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
Filter through 0.45 micron membrane before injection.

Sample Preparation

Accurately weigh 20~40 mg of dried sample (105℃ for 2h) into a 25-mL volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase. Filter through 0.45 μm filter before injection.

Sinochem Qingdao Food Technology Center No. STV-001

Testing Procedure

Assay of Stevia

QA Manager Approval: Wang Zhenyang
R&D Chemist Approval: Jin Yan
Date: June 19, 2006
Updated: Jan 25, 2010

Procedure and Calculation

Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the sample and of the standard.

Measure the peak areas for the seven steviol glycosides from the sample solution (the minor components might not be detected).
Measure the peak areas of stevioside and rebaudioside A from the standard solution.

Calculate the percentage of each of the seven steviol glycosides, X, in the sample from the formula:

$$\%X = [Ws/W] \times [f_x A_x / A_s] \times 100$$

where
Ws is the amount (mg) of stevioside in the standard solution
W is the amount (mg) of sample in the sample solution
As is the peak area of stevioside from the standard solution
Ax is the peak area of X from the sample solution
fx is the ratio of the formula weight of X to the formula weight of stevioside (see the table below)

Peak Sequence	Name	Abbreviation	Relative retention time	Relative formula weight ratio
1	rubusoside	RU	0.12-0.16	0.80
2	dulcoside A	DA	0.25-0.30[a]	0.98
3	steviolbioside	SB	0.35-0.41[b]	0.80
4	stevioside	STV	0.45-0.48[c]	1.00
5	rebaudioside C	RC	0.63-0.69[d]	1.18
6	rebaudioside B	RB	0.73-0.79	1.00
7	rebaudioside A	RA	1.00	1.20

Due to the difference of HPLC condition, especially the difference of columns, some of our actual relative retention times deviate from the data in the above table:
a: 0.22-0.26
b: 0.35-0.39

c: 0.54-0.58
d: 0.70-0.74

Note

If it is required, and to minimize the detecting error, use STV standard to quantify STV in the sample, use RA standard to quantify RA in the sample.

Method Validation Data of Assay of Stevia

Sinochem Qingdao Food Technology Center
September 7, 2010
Chemist: Jin Yan
Manager: Wang Zhenyang

Sample used: Rebaudioside A (97.0%), Control sample(has five glycosides)

1 Accuracy

Three concentration levels, nine samples were prepared.

Sample No.	Sample Weight, mg	Volume, mL	Concentration, mg/mL	Peak Area	Actual content, %	Test content, %	Recovery Percentage, %
A-a1	29.0	25	1.16	1824	97.0	97.1	100.1
A-a2	28.4	25	1.136	1784	97.0	97.0	100.0
A-a3	32.0	25	1.28	2001	97.0	96.6	99.6
A-b1	23.8	25	0.952	1496	97.0	96.9	99.9
A-b2	25.3	25	1.012	1585	97.0	96.6	99.6
A-b3	24.3	25	0.972	1546	97.0	98.1	101.1
A-c1	19.0	25	0.76	1199	97.0	97.0	100.0
A-c2	20.2	25	0.808	1265	97.0	96.3	99.3
A-c3	19.5	25	0.8	1247	97.0	98.3	101.4

Average recovery percentage of level a: 99.9% (within 98.0%~102.0%)
Average recovery percentage of level b: 100.2% (within 98.0%~102.0%)
Average recovery percentage of level c: 100.2% (within 98.0%~102.0%)
RSD of nine Recovery Percentage : 0.70% (<2.0%)
(chromatograms: A-a1,A-a2,A-a3,A-b1,A-b2,A-b3,A-c1,A-c2,A-c3)

2 Repeatability

On the concentration level of 1mg/mL, six samples were tested:

Sample No.	Sample Weight, mg	Volume, mL	Concentration, mg/mL	Peak Area	Content, %
R-1	23.8	25	0.952	1486	96.2
R-2	25.3	25	1.012	1585	96.6
R-3	24.3	25	0.972	1546	98.1
R-4	25.1	25	1.004	1582	97.2
R-5	24.8	25	0.992	1547	96.2
R-6	25.5	25	1.02	1613	97.6

RSD of six content results: 0.79% (<2.0%)
(chromatograms: R-1,R-2,R-3,R-4,R-5,R-6)

3 System suitability

Six replicate injections were carried.

Sample No.	Sample Weight, mg	Volume, mL	Concentration, mg/mL	Peak Area	Retention time, min	Tailing factor
P-1	23.8	25	0.952	1503	22.119	1.289
P-2	23.8	25	0.952	1501	22.102	1.311
P-3	23.8	25	0.952	1518	22.090	1.340
P-4	23.8	25	0.952	1457	22.082	1.266
P-5	23.8	25	0.952	1534	22.054	1.208
P-6	23.8	25	0.952	1486	22.147	1.281

RSD of Peak Area : 1.77% (<2.0%).

RSD of Retention Time : 0.14% (<1.0%)

(chromatograms: P-1,P-2,P-3,P-4,P-5,P-6)

4 Specificity

A control sample which has five steviol glycosides was tested.

And a blank sample was used.

Steviol glycosides	Retention time, min	Resolution	Tailing factor
dulcoside A	5.249	--	1.353
Steviolbioside	8.065	6.1	1.050
Stevioside	12.256	6.08	1.377
rebaudioside C	15.951	3.59	1.606
rebaudioside A	22.009	4.43	1.335

Resolution: >2.0

Tailing factor: <2.0

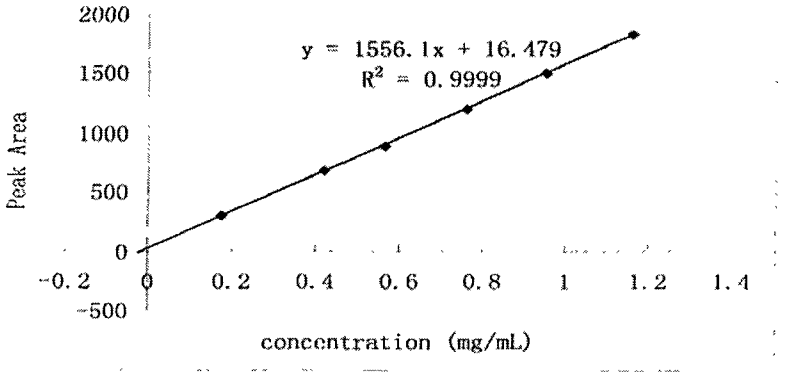
No impurity peaks was found in the blank sample chromatogram.

(chromatograms: Control-1, Control-2, blank)

5 Linearity

A 6-point calibration curve was generated by Concentration (X) and Peak Area (Y).

Sample No.	Sample weight, Mg	Volume, mL	Concentration, mg/mL	Peak Area	Response factor (Peak Area / concentration)
L-1	29.0	25	1.16	1824	1572
L-2	23.8	25	0.952	1496	1571
L-3	19.0	25	0.76	1199	1578
L-4	14.1	25	0.564	888	1574
L-5	10.5	25	0.42	676	1610
L-6	4.4	25	0.176	290	1648



Regression equation: Y=1556.1X+16.479
Correlation coefficient: 0.9999 (>0.998)
RSD of response factor is 1.9% (<2.0%)
y-intercept/ maximum of Y value: 16.479/1824=0.90% (<2.0%)

(chromatograms: L-1 ~ L-6)

Analytical manucript of five batches of stevia (content >95%)

Sinochem Qingdao Food Technology Center
September 7, 2010
Chemist: Jin Yan
Manager: Wang Zhenyang

Sample used:
RA Standard : Rebaudioside A (abbreviation: RA) , min.99.0%, Wako, Lot CDP6587
Control sample.
Five batches of stevia samples:
20081107
20090305
20091011
20100201
20100524

See Figures: RA Standard, Control sample, 20081107, 20090305, 20091011, 20100201, 20100524

Five batches of stevia: Analytical manuscript

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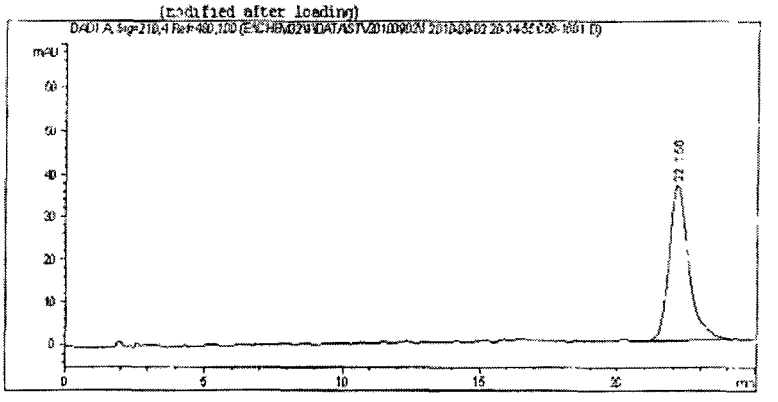
                  (modified after loading)
```



Area Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Saturday, September 25, 2010 4:29:45 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=210,4 Ref=400,100

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   [mAU*s]      %
----|-------|------|-------|----------|--------|--------------------
   1   5.322         0.0000    0.00000   0.0000
   2   8.148         0.0000    0.00000   0.0000
   3  12.320         0.0000    0.00000   0.0000
   4  16.008         0.0000    0.00000   0.0000
   5  22.156 VB      0.7531 1821.24536 100.0000

Totals :                     1821.24536


3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve
```

Instrument 1 10/12/2010 11:03:21 AM JY

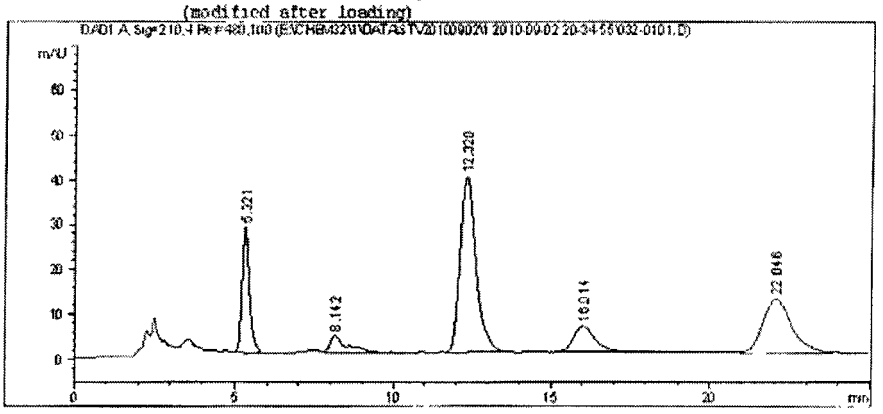
Page 1 of 2

Figure: RA Standard

Five batches of stevia: Analytical manuscript

Data File E:\CHEM32\1\DATA\STV20100902\1 2010-09-02 20-34-55\032-0101.D

```
Acq. Operator   : JY                          Seq. Line :  1
Acq. Instrument : Instrument 1                 Location : Vial 32
Injection Date  : 9/2/2010 8:36:54 PM             Inj :  1
                                               Inj Volume : 10 µl
Acq. Method     : e:\Chem32\1\DATA\STV20100902\1 2010-09-02 20-34-55\STV-LXX.M
Last changed    : 9/2/2010 8:58:56 PM by JY
                  (modified after loading)
Analysis Method : E:\CHEM32\1\METHODS\STV-LXX.M
Last changed    : 10/12/2010 11:00:41 AM by JY
                  (modified after loading)
```



Area Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Saturday, September 25, 2010 4:29:45 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=210,4 Ref=480,100

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   [mAU*s]      %
----|-------|------|-------|----------|--------|--------------------
   1   5.321 VB      0.2515  457.21848  15.0426
   2   8.142 VB      0.5241  149.22571   4.9096
   3  12.320 BB      0.5472 1396.58789  45.9482
   4  16.014 BB      0.6575  278.69211   9.1691
   5  22.046 VB      0.8507  757.76086  24.9306

Totals :                   3039.48505

6 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Invalid calibration curve
```

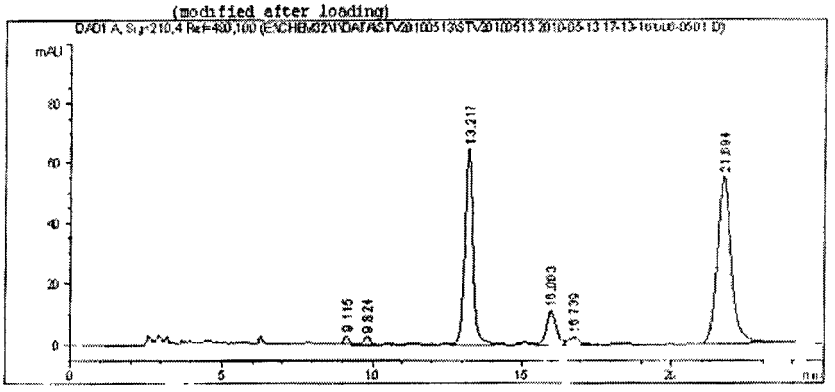
Instrument 1 10/12/2010 11:01:09 AM JY Page 1 of 2

Figure: Control sample

Five batches of stevia: Analytical manuscript

Data File E:\CHEM32\1\DATA\STV20100513\STV20100513 2010-05-13 17 13-16\006-0501.D

```
Acq. Operator    : LAIQY                          Seq. Line :   5
Acq. Instrument  : Instrument 1                    Location : Vial 6
Injection Date   : 5/13/2010 7:08:12 PM                Inj :   1
                                                  Inj Volume : 10 µl
Acq. Method      : e:\Chem32\1\DATA\STV20100513\STV20100513 2010-05-13 17-13-16\STV-LXX.M
Last changed     : 5/13/2010 5:13:14 PM by LAIQY
Analysis Method  : E:\CHEM32\1\METHODS\STV-LXX.M
Last changed     : 10/12/2010 10:40:09 AM by JY
                   (modified after loading)
```



Area Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Saturday, September 25, 2010 4:29:45 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=480,100
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	5.322		0.0000	0.00000	0.0000	
2	8.148		0.0000	0.00000	0.0000	
3	9.115	BV	0.2138	38.04542	1.1343	?
4	9.824	VV	0.2410	42.37178	1.2633	?
5	12.320		0.0000	0.00000	0.0000	
6	13.217	VB	0.2846	1213.85669	36.1905	?
7	16.000	VV	0.3454	250.79462	7.4773	
8	16.739	VB	0.3847	61.63340	1.8376	?
9	21.694	BB	0.4795	1747.37109	52.0970	
Totals :				3354.07300		

```
4 Warnings or Errors :
```

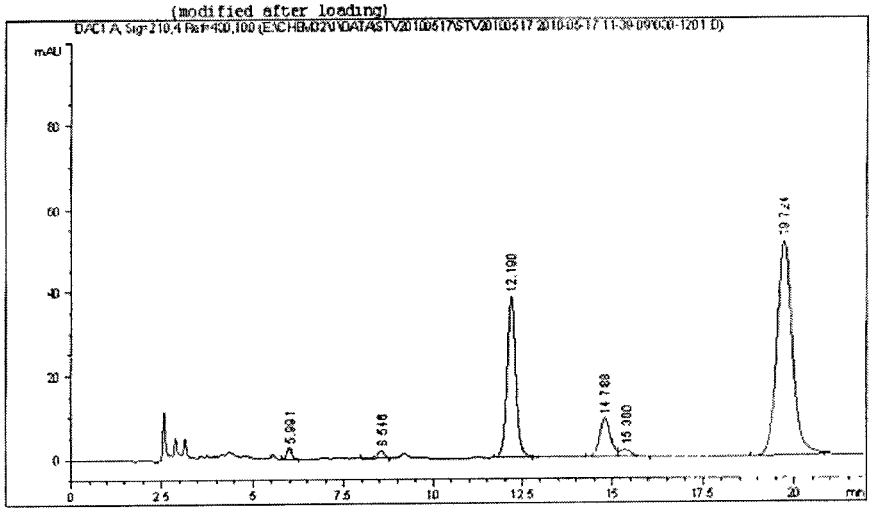
Instrument 1 10/12/2010 10:42:52 AM JY Page 1 of 2

Figure: 20081107

Five batches of stevia: Analytical manuscript

Data File E:\CHEM32\1\DATA\STV20100517\STV20100517 2010-05-17 11-39-09\030-1201.D

```
Acq. Operator   : JY                                  Seq. Line :  12
Acq. Instrument : Instrument 1                       Location : Vial 30
Injection Date  : 5/17/2010 4:03:23 PM                    Inj :  1
                                                    Inj Volume : 10 µl
Acq. Method     : e:\Chem32\1\DATA\STV20100517\STV20100517 2010-05-17 11-39-09\STV-LXX.M
Last changed    : 5/17/2010 1:14:06 PM by jy
Analysis Method : E:\CHEM32\1\METHODS\STV-LXX.M
Last changed    : 10/10/2010 1:22:37 PM by JY
                  (modified after loading)
```



Area Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Saturday, September 25, 2010 4:29:45 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=210,4 Ref=400,100

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   [mAU*s]      %
----|-------|------|-------|----------|--------|--------------------
   1   5.322         0.0000    0.00000   0.0000
   2   5.991 BB      0.1438   26.50507   1.0823 ?
   3   8.148         0.0000    0.00000   0.0000
   4   8.546 BV      0.2356   33.01356   1.3481 ?
   5  12.190 BB      0.2604  651.35931  26.5983
   6  14.788 VV      0.3280  197.82434   8.0782 ?
   7  15.360 VB      0.2899   40.22847   1.6427 ?
   8  16.008         0.0000    0.00000   0.0000
```

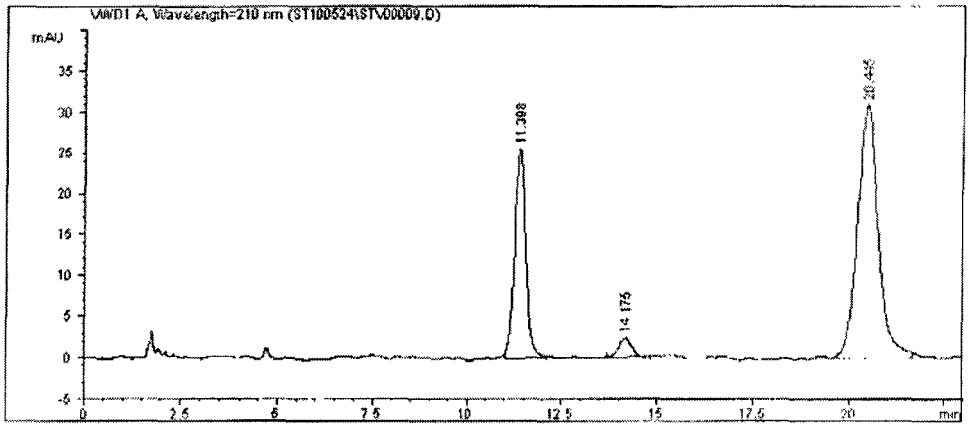
Instrument 1 10/18/2010 1:24:03 PM JY Page 1 of 2

Figure: 20090305

Five batches of stevia: Analytical manuscript

Data File C:\HPCHEM\1\DATA\ST100524\STV00009.D Sample Name: stv

```
Injection Date  : 5/25/2010 12:02:04 PM
Sample Name     : stv                          Location : Vial 1
Acq. Operator   : wzy
Acq. Method     : C:\HPCHEM\1\METHODS\STV9320.M
Last changed    : 5/25/2010 11:58:22 AM by wzy
                  (modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\STV9320.M
Last changed    : 10/18/2010 1:45:28 PM by LM
                  (modified after loading)
tianjutang
```




```
                    Area Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      9/10/2009 2:57:31 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=210 nm

Peak RetTime Type   Width     Area      Area    Name
  #   [min]         [min]  mAU   *s       %
----|-------|------|-------|----------|--------|--------------------
   1   8.569         0.0000     0.00000   0.0000 a
   2  11.398 BB      0.3125   520.75519  30.3284 ?
   3  14.175 BP      0.3577    49.54276   2.8853 ?
   4  20.445 BB      0.5680  1146.75610  66.7863 ?

Totals :                     1717.05405

 Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

                       *** End of Report ***
```

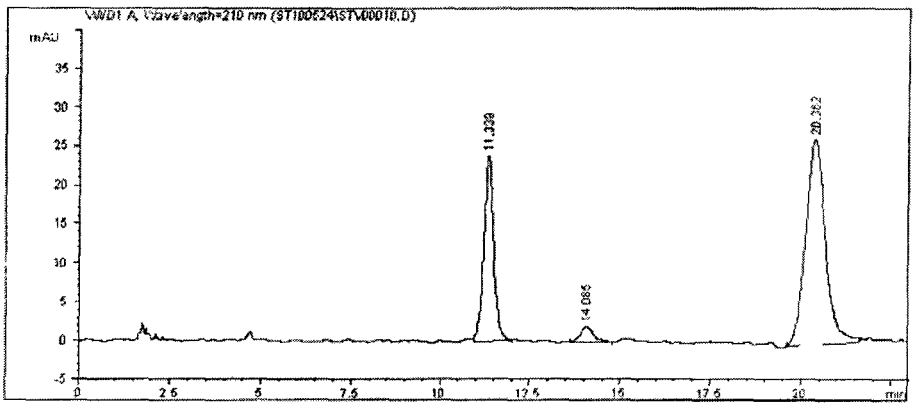
RandD LC 10/18/2010 1:45:37 PM LM Page 1 of 1

Figure: 20091011

Five batches of stevia: Analytical manuscript

Data File C:\HPCHEM\1\DATA\ST100524\STV00010.D Sample Name: stv

Injection Date : 5/25/2010 12:39:42 PM
Sample Name : stv Location : Vial 1
Acq. Operator : wzy
Acq. Method : C:\HPCHEM\1\METHODS\STV9320.M
Last changed : 5/25/2010 12:38:13 PM by wzy
(modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\STVS320.M
Last changed : 10/18/2010 1:46:52 PM by LM
(modified after loading)
tianjutang



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 9/10/2009 2:57:31 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Area %	Name
1	8.569		0.0000	0.00000	0.0000	a
2	11.339	BB	0.3086	474.47192	30.3880	?
3	14.085	BP	0.3896	50.19346	3.2147	?
4	20.362	PB	0.5741	988.99756	63.3412	?
5	29.440	BV	0.6034	47.71770	3.0561	?

Totals : 1561.38065

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

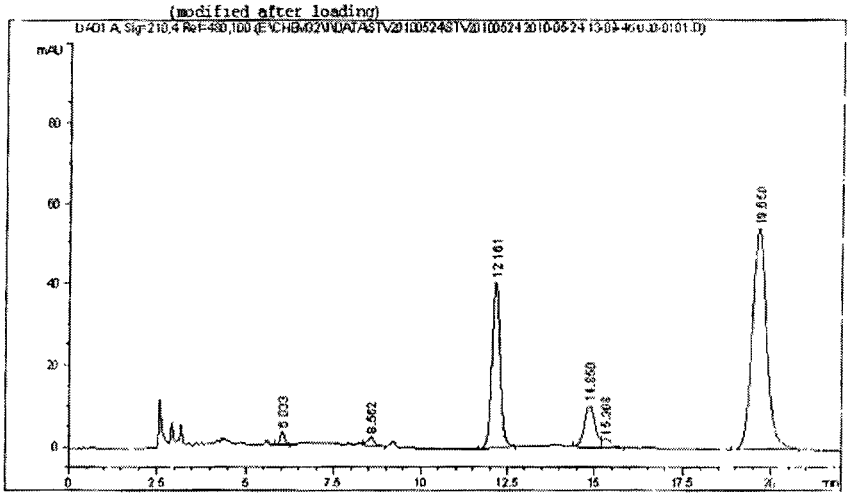
RandD LC 10/18/2010 1:46:59 PM LM Page 1 of 1

Figure: 20100201

Five batches of stevia: Analytical manuscript

Data File E:\CHEM32\1\DATA\STV20100524\STV20100524 2010-05-24 13-09 46\030-0101.D

```
Acq. Operator   : wzy                          Seq. Line :  1
Acq. Instrument : Instrument 1                  Location : Vial 30
Injection Date  : 5/24/2010 1:11:42 PM             Inj :  1
                                               Inj Volume : 10 µl
Acq. Method     : e:\Chem32\1\DATA\STV20100524\STV20100524 2010-05-24 13-09-46\STV-LXX.M
Last changed    : 5/24/2010 12:42:15 PM by wzy
Analysis Method : E:\CHEM32\1\METHODS\STV-LXX.M
Last changed    : 10/18/2010 1:22:37 PM by JY
                  (modified after loading)
```



Area Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      Saturday, September 25, 2010 4:29:45 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=480,100
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	5.322		0.0000	0.00000	0.0000	
2	6.033	BB	0.1263	26.40694	1.0490	?
3	8.148		0.0000	0.00000	0.0000	
4	8.562	VV	0.2193	32.14241	1.2768	?
5	12.161	BB	0.2507	663.55719	26.3592	
6	14.850	BV	0.3194	215.57231	8.5634	?
7	15.308	VB	0.2617	37.35244	1.4838	?
8	16.008		0.0000	0.00000	0.0000	

Instrument 1 10/18/2010 1:26:38 PM JY

Page 1 of 2

Figure: 20100524

Calculate the percentage of each of the seven steviol glycosides, X, in the sample from the formula:

$$\%X = [Ws/W] \times [fxAx/As] \times 100$$

where

Ws is the amount (mg) of rebaudioside A in the standard solution

W is the amount (mg) of sample in the sample solution

As is the peak area of rebaudioside A from the standard solution

Ax is the peak area of X from the sample solution

fx is the ratio of the formula weight of X to the formula weight of rebaudioside A (see "Testing Procedure-Assay of Stevia", the relative formula weight ratio is changed since rebaudioside A standard is used)

Table 1: relative formula weight ratio

Name	Abbreviation	Relative formula weight ratio
dulcoside A	DA	0.817
stevioside	STV	0.833
rebaudioside C	RC	0.983
rebaudioside A	RA	1.000

Table 2: Weights and peak areas

	Weight (dry), mg	DA	STV	RC	RA
RA standard sample	27.0	--	--	--	1821
20081107	45.8	--	1214	251	1747
20090305	34.6	26	651	198	1500
20091011	25.0	--	521	50	1147
20100201	21.8	--	474	50	989
20100524	36.0	26	664	216	1542

Calculate glycosides by the formula, data in table 1 and table 2.

Note: RA standard sample's purity is min. 99.0%, Ws =27.0×0.99 mg

20081107:

STV%=(27.0×0.99/45.8)×(0.833×1214/1821)×100
=32.4

RC%=(27.0×0.99/45.8)×(0.983×251/1821)×100
=7.91

RA% =(27.0×0.99/45.8)×(1.000×1747/1821)×100
=56.0

20090305:

DA%=(27.0×0.99/34.6)×(0.817×26/1821)×100
=0.90

STV%=(27.0×0.99/34.6)×(0.833×651/1821)×100

=23.0

RC%=(27.0×0.99/34.6)×(0.983×198/1821)×100

=8.26

RA% =(27.0×0.99/34.6)×(1.000×1500/1821)×100

=63.6

20091011:

STV%=(27.0×0.99/25.0)×(0.833×521/1821)×100

=25.5

RC%=(27.0×0.99/25.0)×(0.983×50/1821)×100

=2.89

RA% =(27.0×0.99/25.0)×(1.000×1147/1821)×100

=67.3

20100201:

STV%=(27.0×0.99/21.8)×(0.833×474/1821)×100

=26.6

RC%=(27.0×0.99/21.8)×(0.983×50/1821)×100

=3.31

RA% =(27.0×0.99/21.8)×(1.000×989/1821)×100

=66.6

20100524:

DA%=(27.0×0.99/36.0)×(0.817×26/1821)×100

=0.87

STV%=(27.0×0.99/36.0)×(0.833×664/1821)×100

=22.6

RC%=(27.0×0.99/36.0)×(0.983×216/1821)×100

=8.66

RA% =(27.0×0.99/36.0)×(1.000×1542/1821)×100

=62.9

Glycoside distribution/Test results

	DA,%	STV,%	RC,%	RA,%	Total,%
RA standard sample	--	--	--	99.0	99.0
20081107	--	32.4	7.91	56.0	95.6
20090305	0.90	23.0	8.26	63.6	95.8
20091011	--	25.5	2.89	67.3	95.7
20100201	--	26.6	3.31	66.6	96.5
20100524	0.87	22.6	8.66	62.9	95.0

APPENDIX D

Certificates of Analysis of Five Production Batches of Sinochem's SG 95 High Purity Steviol Glycosides

SINOCHEM QINGDAO CO., LTD.

CERTIFICATE OF ANALYSIS

PRODUCT:	STEVIOL GLYCOSIDES 95%
BOTANICAL NAME:	*Stevia Rebaudiana Bertoni*
PART USED:	LEAVES
EXTRACT SOLVENT:	WATER & ETHANOL
SHELF LIFE:	2 YEARS WHEN KEPT IN ORIGINAL PACKAGE
STANDARD:	*PREPARED AT THE 69th JECFA (2008) AND PUBLISHED IN FAO JECFA MONOGRAPHS 5 (2008)*

ITEMS	STANDARD	BATCH NO.					TEST METHOD
		20081107	20090305	20091011	20100201	20100524	
APPERANCE	WHITE TO OFF-WHITE POWDER	COMPLIES	COMPLIES	COMPLIES	COMPLIES	COMPLIES	Visual Check
IDENTIFICATION	PEAK OF STEVIOSIDE & REBAUDIOSDIE A	COMPLIES	COMPLIES	COMPLIES	COMPLIES	COMPLIES	HPLC
ASSAY (steviol glycosides)	NLT 95%	95.6%	95.8%	95.7%	96.5%	95.0%	HPLC
SWEETNESS	200-300	COMPLIES	COMPLIES	COMPLIES	COMPLIES	COMPLIES	GB8270-1999
PH	4.5~7.0	6.1	6.0	5.6	5.9	6.7	USP29 <791>
LOSS ON DRYING	NMT 6.0%	1.85%	3.01%	2.36%	1.45%	2.86%	USP31 <731>
RESIDUE ON IGNITION	NMT 1.0%	0.18%	0.12%	0.05%	0.03%	0.05%	USP31 <281>
METHANOL	NMT 200PPM	<200PPM	<200PPM	<200PPM	<200PPM	<200PPM	GC-MS
ETHANOL	NMT 5000PPM	<5000PPM	<5000PPM	<5000PPM	<5000PPM	<5000PPM	GC-MS
TOTAL HEAVY MEALS	NMT 10PPM	COMPLIES	COMPLIES	COMPLIES	COMPLIES	COMPLIES	USP31 <231>
Pb	NMT 1PPM	COMPLIES	COMPLIES	COMPLIES	NOT DETECTED	NOT DETECTED	ICP MS AOAC
As	NMT 1PPM	COMPLIES	COMPLIES	COMPLIES	NOT DETECTED	NOT DETECTED	ICP MS AOAC
TOTAL PLATE COUNT	< 1000 CFU/G	COMPLIES	COMPLIES	COMPLIES	COMPLIES	COMPLIES	USP31 <2021>
YEAST AND MOLD, CFU/G	< 100 CFU/G	COMPLIES	COMPLIES	COMPLIES	COMPLIES	COMPLIES	USP31 <2021>
E. COLI	NEGATIVE	COMPLIES	COMPLIES	COMPLIES	COMPLIES	COMPLIES	USP31 <2021>
STAPHYLOCOCCUS AUREUS	NEGATIVE	COMPLIES	COMPLIES	COMPLIES	COMPLIES	COMPLIES	USP31 <2021>
SALMONELLA SP.	NEGATIVE	COMPLIES	COMPLIES	COMPLIES	COMPLIES	COMPLIES	USP31 <2021>
PESTICIDES	NOT DETECTED	COMPLIES	COMPLIES	COMPLIES	COMPLIES	COMPLIES	USP31 <467>
QUANTITY:		500kgs	500kgs	500kgs	500kgs	500kgs	

SINOCHEM QINGDAO CO.,LTD.

APPENDIX E

Pesticide Residue Analyses for Sinochem's SG 95 High Purity Steviol Glycosides

SGS

Test Report **No.: QDFDO100805505FD** **Date: Sep 26, 2010**

Client name Sinochem Qingdao Co , Ltd
Client address: The North Building, Golden Plaza, 20 Xianggangzhong Road, Qingdao, 266071, China

The following sample(s) was/were submitted by/ on behalf of the client as:
Sample information (sample name, batch No.,manufacture) stated by the client to be:

Code	SGS job No.	Sample name	Batch No.	Manufacture
#1	QDFDO100805505FD	Stevia leaf	20100806	Jining Stevia Planting Base

SGS reference No SHAFD1012700001
Date of receipt Aug 31, 2010
Testing period Aug 31, 2010 ~ Sep 14, 2010

TEST(S) REQUESTED:
Selected test(s) as requested by applicant:
--To determine the Pesticide Residue Content in the submitted samples, including Organochlorine, Pyrethroid, Organophosphorous, etc

TEST RESULT(S):

Code	Test Items		Test method	Unit	MDL	Test result
1	Alachlor	甲草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
2	Aldrin	艾氏剂	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
3	Benalaxyl	苯霜灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
4	Bendiocarb	恶虫威	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
5	Benfluralin	乙丁氟灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
6	Benfuresate	呋草磺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
7	BHC-a	a-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
8	BHC-β	β-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
9	BHC-r	林丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 10	ND
10	BHC-δ	δ-六六六	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND

SAMPLE DESCRIPTION: Sample in the bag.

Remark:
1 The results apply only to the samples as supplied
2 The test was carried out by a SGS laboratory.
3 ND=Not Detected

Signed for and on behalf of SGS

SGS Center No 143 Zhuzhou Road Hi-Tech Industrial Park,Qingdao,China 266101 t (86-532) 68999106 f (86-532) 83864703
中国·青岛·高科技工业园珠洲路143号通标中心 邮编 266101 t (86-532) 68999106 f (86-532) 83864703

SGS

Test Report No.: QDFDO100805505FD Date: Sep 26, 2010

Code	Test Items		Test method	Unit	MDL	Test result
11	Bifenthrin	联苯菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
12	Bromophos ethyl	乙基溴硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
13	Bromopropylate	溴螨酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
14	Butachlor	丁草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
15	Carbophenothion	三硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
16	Chlorbenside	氯杀螨	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.10	ND
17	Chlordane-cis	顺式-氯丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
18	oxy-Chlordane	氧氯丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
19	trans-Chlordane	反式氯丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
20	Chlorfenapyr	虫螨腈	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
21	Chlorfenson	杀螨酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
22	Chlorfenvinphos	毒虫畏	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.10	ND
23	Chlorobenzilate	乙酯杀螨醇	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
24	Chlorpyrifos	乙毒死蜱	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
25	Chlorpyrifos-methyl	甲基毒死蜱	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
26	Chlozolinate	克氯酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
27	Cyfluthrin	氟氯氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.10	ND
28	λ-Cyhalothrin	λ-氯氟氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
29	Cypermethrin	氯氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.10	ND
30	o,p'-DDD	o,p'-滴滴滴	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
31	p,p'-DDD	p,p'-滴滴滴	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
32	o,p'-DDE	o,p'-滴滴伊	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND

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Test Report No.: QDFDO100805505FD Date: Sep 26, 2010

Code	Test Items		Test method	Unit	MDL	Test result
33	p,p'-DDE	p,p'-滴滴伊	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
34	o,p'-DDT	o,p'-滴滴涕	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
35	p,p'-DDT	p,p'-滴滴涕	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.10	ND
36	Deltamethrin	溴氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
37	Diazinon	二嗪磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
38	Dichlofenthion	除线磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
39	Dicofol	二氯杀螨醇	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
40	Dieldrin	狄氏剂	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.10	ND
41	Dimethenamid	二甲吩草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
42	Dimethylvinphos	甲基毒虫畏	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
43	Diphenamid	双苯酰草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
44	Disulfoton	乙拌磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
45	a-Endosulfan	a-硫丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
46	β-Endosulfan	β-硫丹	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 10	ND
47	Endosulfan sulfate	硫丹硫酸酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
48	EPN	苯硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 10	ND
49	Ethalfluralin	乙丁烯氟灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
50	Ethion	乙硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
51	Ethofumesate	乙氧呋草黄	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
52	Ethoprophos	灭线磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
53	Fenchlorphos	皮蝇磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
54	Fenitrothion	杀螟硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND

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Test Report **No : QDFDO100805505FD** **Date: Sep 26, 2010**

Code	Test Items		Test method	Unit	MDL	Test result
55	Fenpropathrin	甲氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
56	Fensulfothion	丰索磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
57	Fenthion	倍硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
58	Fenvalerate	氰戊菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
59	Flusilazole	氟硅唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
60	tau-Fluvalinate	氟胺氰菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
61	Fonofos	地虫硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
62	Furalaxyl	呋霜灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
63	Heptachlor	七氯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
64	Heptenophos	庚烯磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
65	Hexachlorobenz one	六氯苯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
66	Isofenphos	异柳磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
67	Metalaxyl	甲霜灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
68	Methyl-pentachlorophe nyl sulfide	甲基五氯苯硫醚	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
69	Napropamide	敌草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
70	Nitrothal-isopropyl	稻瘟酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
71	Paclobutrazol	多效唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 10	ND
72	Penconazole	戊菌唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
73	Pendimethalin	甲戊乐灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
74	Pentachloroanili ne	五氯苯胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
75	Pentachloroanis ole	五氯茴香醚	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
76	Permethrin	氯菊酯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND

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Code	Test Items		Test method	Unit	MDL	Test result
77	Phenthoate	稻丰散	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
78	Phorate	甲拌磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
79	Pirimiphos-ethyl	乙基嘧啶磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
80	Pirimiphos-methyl	甲基嘧啶磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
81	Procymidone	腐霉利	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
82	Profenophos	丙溴磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
83	Prometryn	扑草净	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
84	Propargite	炔螨特	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
85	Propiconazole	丙环唑	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 10	ND
86	Propyzamide	炔苯酰草胺	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
87	Quinalphos	喹硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
88	Quintozene	五氯硝基苯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
89	Safrotin	三唑磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0.01	ND
90	Salithion	蒜果磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
91	Sulfotep	治螟磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
92	Tebuconazole	戊唑醇	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
93	Tecnazene	四氯硝基苯	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
94	Terbufos	特丁硫磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
95	Tetrachlorvinphos	杀虫畏	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
96	Tetradifon	四氯杀螨砜	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
97	Tolclofos-methyl	甲基立枯磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND

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Code	Test Items		Test method	Unit	MDL	Test result
98	Triazophos	三唑磷	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND
99	Trifluralin	氟乐灵	With reference to US FDA PAM, GB/T 19648-2006	mg/kg	0 01	ND

Code	Test Items		Test method	Unit	MDL	Test result
1	Acetochlor	乙草胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
2	Aldoxycarb	铁灭威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
3	Aramite	杀螨特	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
4	Anilofos	莎稗磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
5	Azinophos methyl	保棉磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
6	Azoxystrobin/Pyroxystrobin	肟菌酯/苯菌酯	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
7	Bensulfuron-methyl	苄嘧磺隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
8	Bitertanol	联苯三唑醇	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
9	Buprofezin	噻嗪酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
10	Carbaryl	甲萘威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
11	Carbofuran	虫螨威/克百威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
12	Carbofuran-3-hydroxy	3-羟基虫螨威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
13	Chlorbufam	氯草灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 10	ND
14	Chloroxuron	枯草隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
15	Clethodim	烯草酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 10	ND

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Code	Test Items		Test method	Unit	MDL	Test result
16	Cyanazine	草净津	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
17	Cyprodinil	嘧菌环胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
18	Dichlorvos	敌敌畏	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 10	ND
19	Diethofencarb	乙霉威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
20	Difenoconazole	苯醚甲环唑	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
21	Dimethoate	乐果	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
22	Dimethomorph	烯酰吗啉	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
23	Edifenphos	克瘟散	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
24	Esprocarb	戊草丹	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
25	Ethiofencarb	乙硫甲威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
26	Fenoxycarb	苯氧威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
27	Fenpropimorph	丁苯吗啉	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 10	ND
28	Fentrazamide	四唑嘧酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
29	Haloxyfop-ethoxyethyl	氟吡乙禾灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
30	Haloxyfop-methyl	氟吡甲禾灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 10	ND
31	Hexythiazox	噻螨酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
32	Imazalil	抑霉唑/烯菌灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
33	Iprovalicarb	丙森锌	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 10	ND
34	Isoprocarb	异丙威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
35	Isoprothiolane	稻瘟灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
36	Isoproturon	异丙隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND
37	Lenacil	环草定	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0 01	ND

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Code	Test Items		Test method	Unit	MDL	Test result
38	Linuron	利谷隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.10	ND
39	Malathion	马拉硫磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
40	Mefenacet	本吡醚草胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
41	Methamidophos	甲胺磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.10	ND
42	Methidathion	杀扑磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
43	Methiocarb	久杀威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
44	Metolachlor	异丙甲草胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.10	ND
45	Mevinphos	速灭磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
46	Monocrotophos	久效磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
47	Nicosulfuron	烟嘧磺隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
48	Omethoate	氧化乐果	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
49	Oxadixyl	噁霜灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
50	Oxadizon	恶草酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.10	ND
51	Oxamyl	杀线威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
52	Oxydemeton-methyl	砜吸磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
53	Parathion	对硫磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
54	Phosalone	伏杀硫磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
55	Phosmet	亚胺硫磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.10	ND
56	Pirimicarb	抗蚜威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
57	Prochloraz	咪鲜胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
58	Promecarb	猛杀威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
59	Propamocarb	霜霉威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.10	ND
60	Propoxur	残杀威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND

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Code	Test Items		Test method	Unit	MDL	Test result
61	Pymetrozin	抗蚜酮	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.10	ND
62	Pyrazophos	吡菌磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
63	Pyrimethanil	嘧霉胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
64	Quizalofop-ethyl	天单克	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
65	Rimsulfuron	砜嘧磺隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
66	Spinosad	多杀菌素	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
67	Spiroxamine	（含物和制备杀菌剂）	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
68	Tebufenozide	虫酰肼	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
69	Tebufenpyrad	吡螨胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
70	Thiabendazole	噻菌灵	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
71	Thiacloprid	噻虫啉	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
72	Thifensulfuron-methyl	噻吩磺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
73	Thiodicarb	硫双威	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
74	Thiofanox-sulfone	久效威砜	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
75	Thiofanox-sulfoxid	久效威亚砜	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
76	Tolfenpyrad	唑虫酰胺	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
77	Triasulfuron	醚苯磺隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
78	Triflumizole	氟菌唑	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
79	Triflusulfuron-methyl	氟胺磺隆	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND
80	Vamidothion	完火硫磷	With reference to US FDA PAM GB/T 20769-2008	mg/kg	0.01	ND

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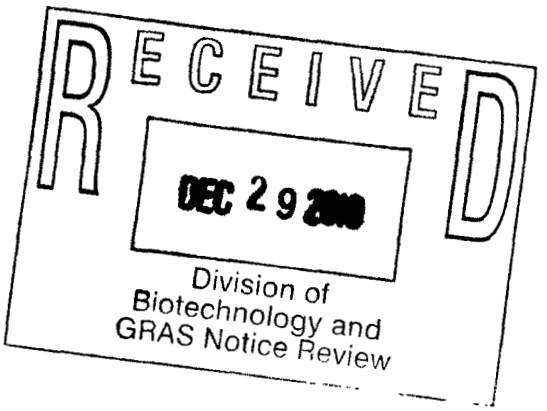
*** End of Report**

SGS Center, No 117 Zhuzhou Road Hi-Tech Industrial Park Qingdao China 266101 t (86-532) 68999136 f (86-532) 83834538 www.cn.sgs.com
中国·青岛 高科技工业园株洲路143号通标中心 邮编 266101 t (86-532) 68999136 f (86-532) 83834538 e sgs.china@sgs.com

GRAS ASSOCIATES, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com

December 22, 2010



Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Robert L. Martin

Re: Revision to Section I.A GRAS Notification
Sinochem High Purity Steviol Glycosides

Dear Dr. Martin:

In response to the agency request that we designate more clearly that the GRAS determination in the above-referenced GRAS notification has been made by the notifying company and as the agent acting on behalf of Sinochem Qingdao Co., we offer the attached replacement page 5.

I hope that this modification responds appropriately to the matter as identified by FDA. If there are any remaining questions, please feel free to contact me for prompt resolution.

Thank you.

(b) (6)

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

Enclosures: Section I.A – Sinochem Qingdao Co. (3 copies)

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

Sinochem Qingdao, Co., Ltd. ("Sinochem") has determined that its high purity steviol glycosides, with rebaudioside A and stevioside as the principal components---which is referred to as SG 95 and by the trade name IceVia™---and which meets the specifications for high purity steviol glycosides (≥ 95%) as described below, is Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS conclusion is based on scientific procedures as described in the following sections; and the evaluation accurately reflects the conditions of the stevia-derived sweetener's intended use in foods.

Signed:

(b) (6)

December 10, 2010

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR97702-3074

Date

B. Name &Address of Notifier

Sinochem Qingdao Co., Ltd.
The North Building, Golden Plaza
20 Xianggang Zhong Road
Qingdao, China 266071

As the notifier, Sinochem accepts responsibility for the GRAS determination that has been made for the high purity steviol glycosides as described in the subject notification; consequently, the high purity steviol glycosides preparations meeting the conditions described herein are exempt from premarket approval requirements for food ingredients.

[1] See 62 FR 18938 (17 April 1997) which is accessible at http://www.gpo.gov/fdsys/pkg/FR-1997-04-17/html/97-97-9706.htm.

SUBMISSION END